University of Alberta
Printing Department

Digitized by the Internet Archive
in 2019 with funding from
University of Alberta Libraries

https://archive.org/details/Husain1965

Thesis
1965
#33

THE UNIVERSITY OF ALBERTA

SULPHUR ISOTOPE FRACTIONATION BY SALMONELLA

by

SHEIKH ANSAR HUSAIN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PHYSICS

EDMONTON, ALBERTA

APRIL, 1965.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled SULPHUR ISOTOPE FRACTIONATION BY SALMONELLA, submitted by Sheikh Ansar Husain in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Significant alternations of the S^{34}/S^{32} ratio in the biological sulphur cycle in nature have been observed with a number of organisms.

In this thesis, reduction of sulphite by Salmonella has been studied. This study differs from previous investigations in that the genus is enteric and has its normal habitat within animal hosts.

In a general study in which the initial conditions of the media were unaltered, enrichments of S^{32} in the product H_2S over the substrate sulphite of as high as 3.4 per cent have been observed with various species of Salmonella. Under anaerobic conditions, the reduction rates were higher than aerobically. The isotope fractionation obtained anaerobically was usually higher than aerobically.

Continuous feeding experiments provided more conclusive results concerning the dependence of the isotope fractionation on reduction rate.

k_1/k_2 values for the competitive reactions

$$S^{32}O_3^{=} \xrightarrow{k_1} H_2S^{32}$$

$$S^{34}O_3^{=} \xrightarrow{k_2} H_2S^{34}$$

of as high as 1.04 were realized with Salmonella heidelberg

in the continuous experiments. In any given reduction, the rate reasonably obeys first order reaction kinetics. The isotope fractionation is found to be inversely proportional to the rate of reduction in agreement with similar observations made by workers with other microorganisms.

ACKNOWLEDGEMENTS

I take this opportunity to extend my sincere thanks to my supervisor, Dr. H. R. Krouse, for suggesting this problem and for his able guidance during the entire work. The help rendered by Mr. R. G. L. McCready and Dr. J. N. Campbell of the Department of Microbiology for the bacteriological part of the work is also gratefully acknowledged.

My thanks are due to the Government of Pakistan in general and the Government of Canada in particular for the arrangement of scholarship under the Colombo plan for my studies here in Canada.

The technical services of Mr. H. McCullough and Mr. Peter Alexander are greatly appreciated. The assistance of Miss H. Hufnagel in the preparation of this manuscript is also acknowledged.

CONTENTS

Page

I. INTRODUCTION

General 1

Equilibrium Isotope Effects 2

Kinetic Isotope Effects 3

Variation of Isotope Abundances in Nature 4

Sulphur Isotope Fractionation Studies 5

Salmonella 13

II. THEORY 14

III. EXPERIMENTAL

Microbiological Reduction of Sulphite 23

Preparation of Sulphur Dioxide (SO_2) Samples for Mass Spectrometric Analysis 25

Mass Spectrometry 28

IV. RESULTS AND DISCUSSION

General Survey 32

Continuous Feeding Experiments 34

Reduction of Sulphate Study 42

V. BIBLIOGRAPHY 43

LIST OF FIGURES

		Page
Figure I	Sulphur Isotope Distribution in Nature	5a
Figure II	Simplified Sulphur Cycle in Nature	8a
Figure III	Sulphur Isotope Fractionation in the Sulphur Cycle (Kaplan and Rittenberg)	11a
Figure IV	Apparatus for Microbiological Reduction of Sulphite	23a
Figure V	Furnace for the Burning of Sliver Sulphide Samples	25a
Figure VI	Sample Handling System on Mass Spectrometer	28a
Figure VII	Sulphite Reduction by Salmonella Species. Aerobic - Anaerobic Comparisons (Initial Conditions unaltered)	32a
Figure VIII	Population Determination (Initial Conditions Unaltered)	33a
Figure IX, & IX(a)	Continuous Reduction of Sulphite by Salmonella heidelberg. BShAn-8 Series	36a
Figure X, & X(a)	Continuous Reduction of Sulphite by Salmonella heidelberg. BShAn-1 Series	37a
Figure XI, & XI(a)	Continuous Reduction of Sulphite by Salmonella heidelberg. BShA-7 Series	38a
Figure XII	Population determination (Continuous Feeding)	38c

LIST OF TABLES

		Page
Table I	Sulphite Reduction with *Salmonella* Species. Aerobic - Anaerobic Comparisons (Initial Conditions Unaltered)	32b
Table II	Continuous Reduction of Sulphite with *Salmonella heidelberg* (37°C, 150 µg. glucose q. 6 hr. anaerobic)	36b
Table III	Continuous Reduction of Sulphite with *Salmonella heidelberg* (37°C, 150 µg. glucose q. 6 hr. anaerobic)	37b
Table IV	Continuous Reduction of Sulphite with *Salmonella heidelberg* (37°C, 150 µg. glucose q. 6 hr. aerobic)	38b

INTRODUCTION

General

At the turn of the century investigations of the radiations emitted from radioactive substances indicated that pairs of inseparable atomic species existed with supposedly identical chemical properties. For example the existence of "Ionium" which was chemically similar to thorium was reported in 1906 by Boltwood (1,2). Soddy (3) found many other examples and in 1910 named those species which were chemically similar but different in their radioactive properties, "isotopes". Isotopes are in fact different nuclear forms of the same element and occupy the same place in the periodic table. Isotopes for the elements of low atomic weight were first demonstrated by J. J. Thomson (4) in 1913 with his positive ray parabola apparatus when he identified two of the three stable isotopes of neon (Ne^{20} and Ne^{22}).

Aston (5,6,7) and Dempster (8,9,10) subsequently made abundance determinations of the isotopes of a large number of elements with their mass spectrographs. Isotopes of some of the elements (carbon, nitrogen, oxygen) were found by optical spectroscopy, (King and Birge (11), Giaque and Johnston (12), Naude (13) and Urey et al (14)).

Since the pioneer work of Aston and Dempster many refinements have been introduced into isotope analyses so that the masses of isotopes can be determined to eight significant figures and differences in isotopic abundance ratios of 0.01 per cent can be detected.

Equilibrium Isotope Effects

Attempts to separate the isotopes by chemical methods initially were unsuccessful and so it was felt that isotopes of an element were identical chemically. This concept changed with the discovery of deuterium by Urey et al (14) in 1931. In view of the fact that deuterium has twice the mass of protium it seemed unreasonable that they should be identical in their chemical behaviour.

Theoretical calculations based on statistical mechanics for the equilibrium constant at different temperatures for the isotope exchange reaction:

$$H_2 + D_2 \rightleftharpoons 2HD$$

were made by Rittenberg and Urey (15) in 1934. It was found that the equilibrium constants differ from unity indicating differences in chemical behaviour of the hydrogen isotopes. Similar studies by Farkas and Farkas (16)

were made for the hydrogen-water exchange reaction. The agreement between theoretical predictions and experimental results was good. Urey and Grieff (17) worked out the equilibrium constants for isotopic exchange of many of the lighter elements. (Boron, Carbon, Oxygen, Nitrogen and Chlorine). These calculations predicted equilibrium isotope effects of as high as 10 per cent for reactions involving these isotopes. The data for many isotope effects have been summarized by Urey (18).

Kinetic Isotope Effects

The discovery of deuterium also led investigators to carry out experiments to show the existence of kinetic isotope effects i.e. different rates of reaction for isotopic atoms and molecules. Partially electrolysed water was shown by Washburn and Urey (19) in 1932 to be richer in deuterium since protium was evolved faster at the cathode. This process was later used for the separation of hydrogen isotopes. Eyring (20) in 1933 pointed out that the greater zero-point vibrational energy of the lighter isotopic bonds caused them to rupture more readily.

Bach et al (21) showed that the rate of reaction of protium with bromine was over three times faster than that of deuterium with bromine. Similar results with the

photochemical reaction of hydrogen and deuterium with chlorine were reported by Farkas and Farkas (22) in 1934. Reviews of kinetic isotope effects with hydrogen have been given by Urey and Teal (23) and Eidinoff (24).

Kinetic isotope effects for elements other than hydrogen were not reported until 1949. Lindsay et al (25) investigated the decomposition of oxalic acid and found that C^{12} bonds rupture more readily than those of C^{13}. Since then kinetic isotope effects of the order of 1 to 10 per cent have been reported for the elements oxygen, nitrogen, sulphur (see review article by McMullen and Thode (26)), selenium (Krouse and Thode (27)), and germanium (Brown and Krouse (28)). An important aspect of the work in kinetic isotope effects is its use in understanding various reaction mechanisms.

Variations of Isotope Abundances in Nature

Many of the isotope fractionations realized in laboratory reactions have also been found to occur in nature. Emeleus et al (29) found the density of natural water samples to vary with geographical location. This variance was attributed to variations in the isotopic composition of hydrogen. Oxygen isotopes (Dole (30,31)) and carbon isotopes (Nier and Gulbransen (32) and Murphy

and Nier (33)) were also found to vary in their abundances and these variations have been related to physical, chemical and biological processes. The stable isotopes of numerous other elements have been found to vary in their natural abundance. Of particular interest to the present study is the variation found in S^{34}/S^{32} ratio in natural and laboratory fractionation studies.

Sulphur Isotope Fractionation Studies

The study of sulphur isotopes is important in natural isotope fractionation investigations because of the existence of sulphur in different valence states and its wide distribution in the earth's crust. Initial data on the sulphur isotope fractionation was published by Thode et al (34). It has been subsequently found that the S^{34}/S^{32} and S^{33}/S^{32} ratios for sulphur vary by about 8 and 4 per cent respectively depending on its origin. The distribution of sulphur isotopes in nature is shown in figure I in which the δ - $^{o}/oo$ value is defined as:

$$\delta(^{o}/oo) = [\frac{S^{34}/S^{32}(\text{Sample})}{S^{34}/S^{32}(\text{Meteorite})} - 1] \times 1000 .$$

The following inferences can be drawn from this distribution:

1. The S^{34}/S^{32} ratio for meteorites is remarkably constant. Igneous sulphides are fairly close to the meteoritic

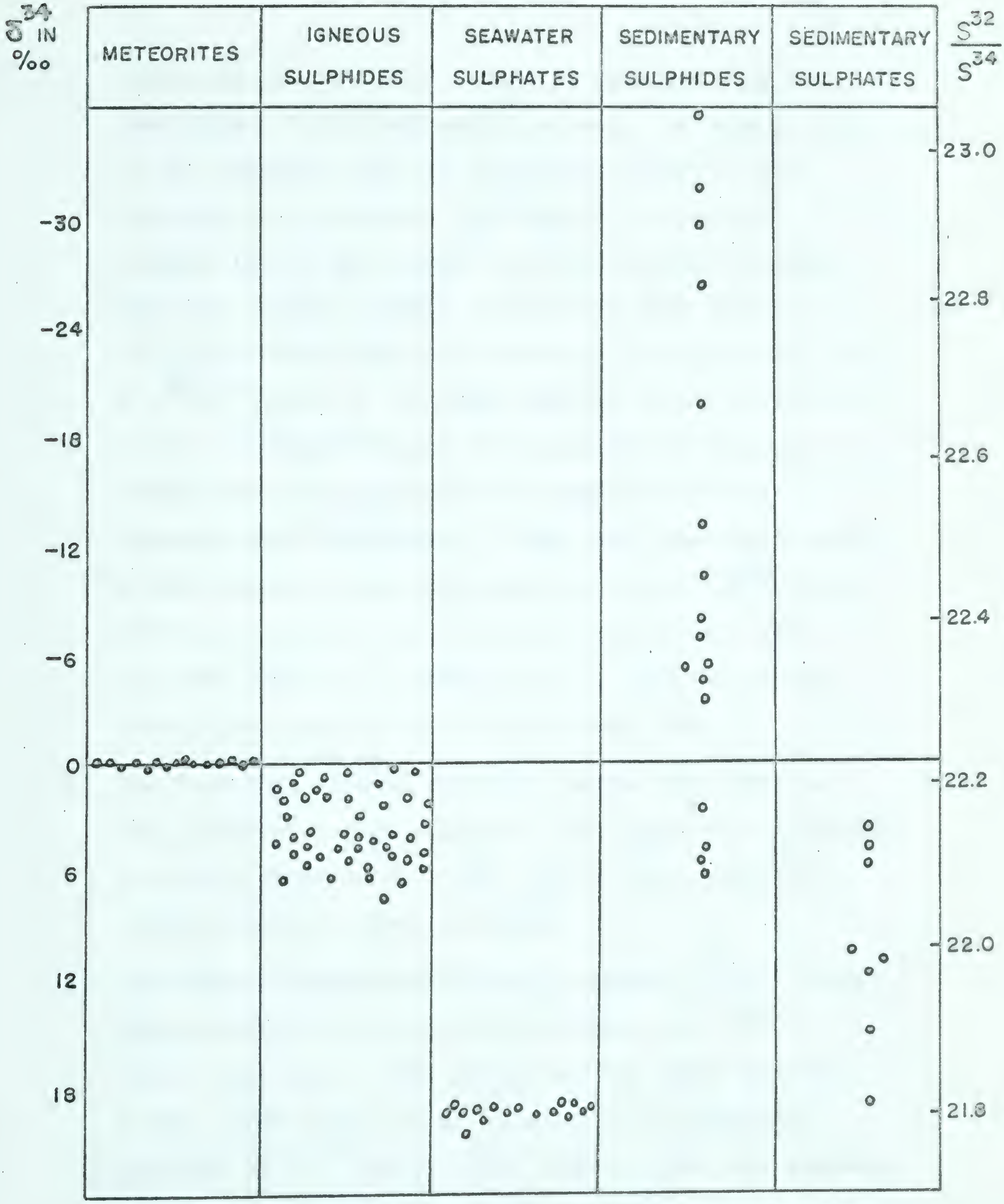


FIG. I SULPHUR ISOTOPE DISTRIBUTION IN NATURE

value and the meteoritic value is approximately the mean value of the terrestrial spread. In view of this it is suggested that the meteoritic S^{34}/S^{32} value represents a primordial abundance of terrestrial sulphur before any process of fractionation occurred. Moreover igneous sulphur should have less chance to be fractionated since its formation, and it should have a S^{34}/S^{32} value in the neighbourhood of the meteoritic value. Although some of the sulphides of igneous origin show an appreciable enrichment in S^{34} as compared with meteorites, it has been found that quite a good number of igneous sulphides have S^{34}/S^{32} values within 0.1 per cent of the meteoritic value. This has been verified by McNamara et al (35), Vinogradov et al (36), Kulp et al (37) and Sakai (38).

2. The fact that igneous sulphides values are close to the meteoritic value suggests high temperature physical processes which do not alter the isotopic ratio as significantly as other processes.

3. Sea water sulphates have a nearly constant S^{34}/S^{32} ratio being enriched in the heavier isotope, i.e. S^{34} by about 2 per cent. This means that the ocean provides a very large reservoir of sulphate at a relatively constant S^{34}/S^{32} level. This suggests that the sulphate ion is well mixed in the oceanic waters.

4. Most of the sedimentary sulphides are seen to be enriched in the lighter isotope i.e. S^{32}, while the sedimentary sulphates are generally enriched in the heavier isotope S^{34}. Since these differences are found to be large, they correspond to low temperature processes which may involve the breaking of chemical bonds.

In order to answer the question of how the distribution of sulphur in figure I has occurred Tudge and Thode (39) calculated the values of the exchange constants for the isotopic exchange reaction:

$$S^{32}O_4^{=} + H_2S^{34} \rightleftharpoons S^{34}O_4^{=} + H_2S^{32}$$

The equilibrium constant for this reaction at 25°C was found to be 1.072 meaning that there should be 7.2 per cent more S^{34} in sulphate than in the hydrogen sulphide when the equilibrium is established. It is in fact interesting to note that the over all percentage spread in the S^{34}/S^{32} ratio is of the same order as the equilibrium constant for this isotopic exchange reaction.

Since the above exchange has not been affected directly under normal conditions it was suggested by Szabo et al (40) that perhaps the well known biological

cycle of sulphur in nature of figure II might be a means of fractionation of sulphur isotopes in view of the above reaction. In this biological sulphur cycle, sulphate is being continuously reduced either by bacterial reduction under anaerobic conditions or through plant metabolism, while the sulphides and H_2S are being oxidized to free sulphur and sulphides.

One of the direct verifications for sulphur isotope fractionation in this cycle is the result of investigations of the Cyrenaican lakes in Africa where $SO_4^{=}$ in solution is microbiologically reduced to H_2S. Oxidizing bacteria also abound in these lakes and oxidize H_2S in solution to elemental sulphur, which is precipitated as a layer at the bottom. In 1951 McNamara and Thode (40) found the elemental sulphur produced at the bottom of these lakes to have S^{34} content about 3.2 per cent less than that of the soluble sulphate from the same lake.

Another striking evidence of the biological sulphur cycle responsible for sulphur isotope fractionation was found in the sulphur wells of Louisiana and Texas. Elemental sulphur is found in calcium carbonate in these deposits. Reduction of the surrounding gypsum to form the H_2S and S^0 had two possible mechanisms:

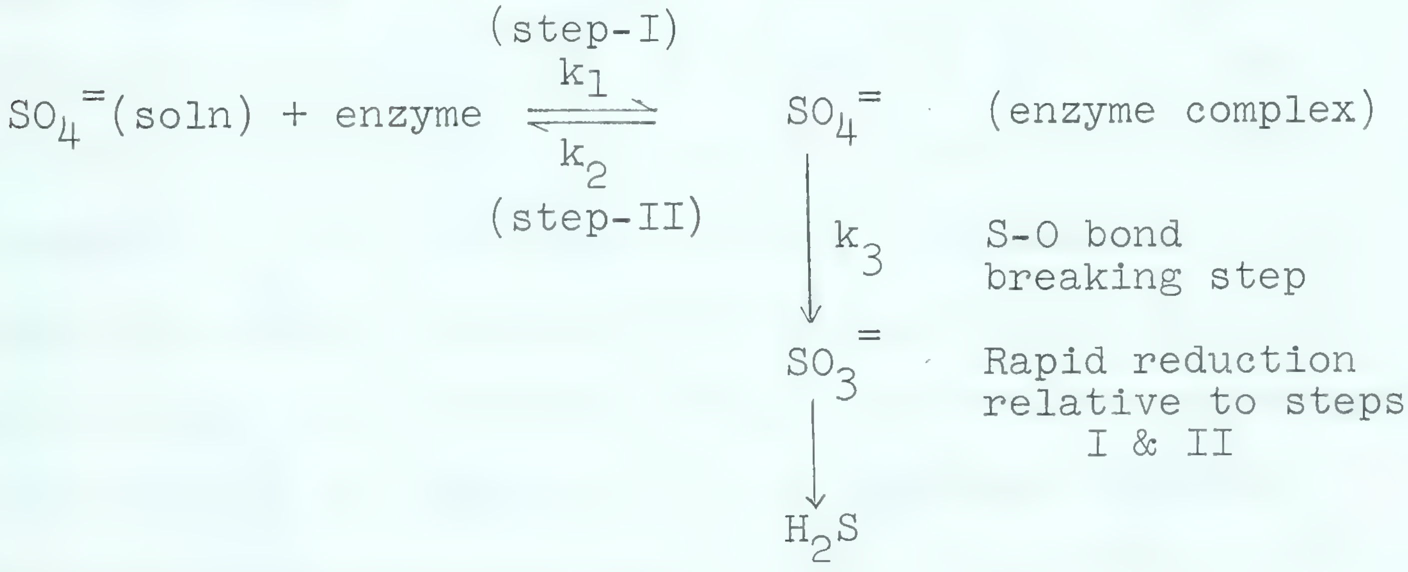
i. The organic reduction of sulphate at high temperatures since oil is found around the periphery of salt domes.

ii. The reduction of sulphate by sulphur bacteria. The petroleum present would supply a source of energy for the bacteria.

In an attempt to establish the nature of the process Thode et al (42) investigated the S^{32}/S^{34} content of the gypsum and the sulphur and sulphide. The S^{34} content of the sulphate was about 4 per cent higher than the elemental sulphur and 5 per cent higher than the sulphides in these salt domes. The first high temperature process is incapable of producing such large isotopic fractionations, whereas the second low temperature process of bacterial reduction can do so.

Microbiological sulphur isotope fractionation in the laboratory was demonstrated in 1951 by Thode et al (43). They reported one per cent depletion of S^{34} in hydrogen sulphide produced in the reduction of sulphate by Desulphovibrio desulphuricans. Jones et al (44) reported a large temperature coefficient for such isotope fractionation. According to their investigations the degree of fractionation could be increased to 25 $^{o}/oo$ by using a higher sulphate concentration and reducing the temperature to about 14 to 20°C. In this way the enrichment is controlled by the temperature regulation

of the rate of reduction. Kaplan et al (45) also reported that sulphate concentration plays a minor role while the rate of reduction of sulphate was the main factor responsible for the degree of isotopic enrichment. A detailed analysis of the phenomenon was carried out by Harrison and Thode (46). Their results indicated variations in the isotope effect of 0 to 2.5 per cent. The degree of fractionation was inversely proportional to the reduction rate, which in turn was controlled by temperature and electron donor concentration. The influence of sulphate was effective only at limiting concentrations. In order to explain the magnitude and direction of isotope effect they have suggested the possibility of two step process of the following nature:



In this reaction mechanism the first step is assumed to involve very little or no isotope effect. The sulphur oxygen bond breaking step involves an isotope effect of about 2.3 per cent as reported by Harrison and Thode (47)

in their chemical reduction of sulphate studies. Thus it is evident that the isotope effect for the entire process will vary considerably depending upon whether step-I or II is a rate controlling step. When both of these steps are nearly equally effective intermediate isotope effects are expected.

Recently Kaplan and Rittenberg (48,49) have extended the studies of isotope fractionation in the natural sulphur cycle. They studied the oxidation of sulphide using chemosynthetic as well as photosynthetic sulphur bacteria and found elemental sulphur, sulphate and an unidentified polythionate as resulting products in the metabolism. In the chemosynthetic process the elemental sulphur was found to be slightly enriched in S^{32} and the sulphate was enriched in S^{32} by as much as 15 $^{o}/oo$. The intermediate polythionate however was always enriched in S^{34} relative to the starting sulphide. The photosynthetic process of sulphide oxidation showed similar trends except that no significant difference was found in the composition of the sulphate in comparison to the reactant sulphide. These fractionation patterns by biological processes in the sulphur cycle are shown in figure III. In this figure the isotope enrichment of the final and intermediate products is designated by 32 and 34 while no fractionation is designated by 'N'.

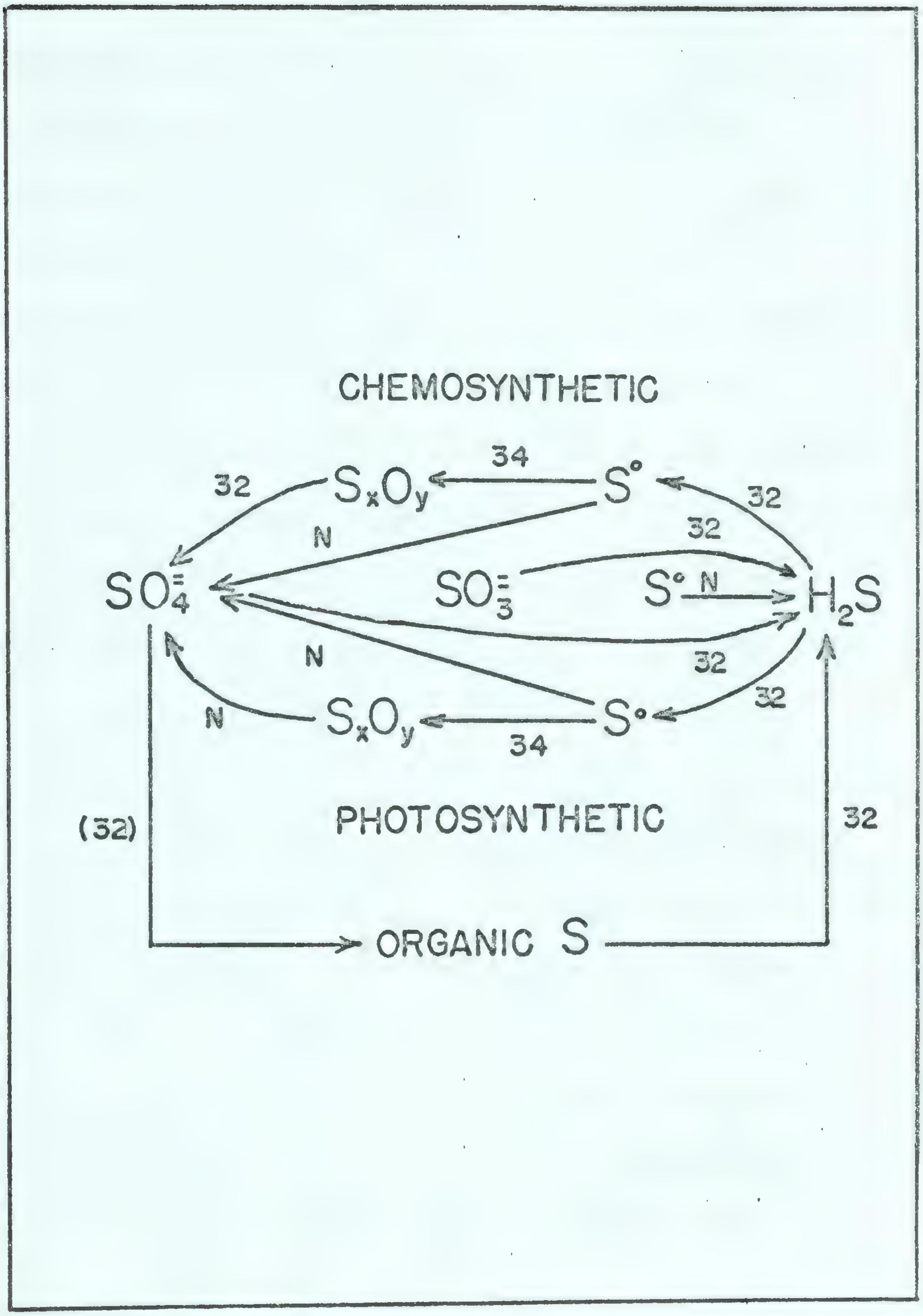


FIGURE III (from KAPLAN and RITTENBERG (48))

Nakai and Jensen (50) have recently conducted reduction of sulphate experiments with natural cultures of marine muds. A constant value of about 1.020 for the ratio of the isotopic reduction rate constants k_1/k_2 was realized and the reaction followed first order kinetics. Moreover they have also demonstrated that the fractionation factors (the ratio S^{32}/S^{34} sulphide//S^{32}/S^{34} Sulphate) exceeded k_1/k_2 and reached 1.043 to 1.062 during the process of reduction in a limited supply of sulphate. This fractionation factor is dependent upon the percentage of reaction and tends to infinity in the neighbourhood of 100 per cent reaction. If the supply is infinite, the fractionation factor retains the value k_1/k_2. This will be mathematically demonstrated in the theory. Also in oxidation experiments with mixed cultures Nakai and Jensen (50) reported the rates of oxidation of S^{32} and $FeS_2{}^{32}$ to be 0.1 per cent faster than the rates for S^{34} and $FeS_2{}^{34}$. The fractionation factors observed between reactants and sulphate produced were found to vary between 1.0003 to 1.0017, indicating more enrichment of S^{32} in sulphate than in sulphur or sulphide.

In this thesis, the reduction of sulphite ion to H_2S has been studied using Salmonella species. This organism is enteric in contrast to other organisms used

in isotope fractionation studies. It was interesting to see therefore if isotope fractionation could be realized with a genus whose normal habitat is within an animal host.

Salmonella

In bacteriology Salmonella are described as Gram-negative non-sporulating rods, of dimensions (1-3 x 0.5-0.7) microns. These are commonly distributed in man, mammals and birds. This genus is easily cultivable both aerobically and anaerobically on common laboratory media. Most of the species of Salmonella produce CO_2 from glucose and H_2S production often occurs from sulphur containing compounds. Their normal habitat is enteric i.e. the Salmonella group are found mainly in the intestine and are common pathogens of mammals.

THEORY

In order to explain the problem of relative reaction rates of isotopic molecules Bigeleisen (51) has developed a theory of the kinetic isotope effect. His theory is essentially based on the "theory of absolute rates" given by Eyring (52) and Evans and Polanyi (53) from the statistical view point.

The theory of absolute rates involves an "activated complex" between the reactant and product. The motion of the nuclei during a chemical reaction is determined by a potential energy surface which is in fact a plot of the potential energy of a system of atoms in their lowest quantum state (electronic) versus the separation between the nuclei. For different degrees of freedom consistent with a particular position on the potential energy surface the moving system of atoms will have a quantitized kinetic energy. The minima on the potential surface correspond to compounds, the compounds being more stable for the lower minima.

A system passing from a particular low region to another corresponds to a reaction. In view of the Boltzman factor the thermal reactions will occur by virtue of the barrier between the reactant and the product. The highest

energy point on this lowest barrier is the activated state and the system at this point is termed as the "activated complex". The highest point is a sort of saddle point with positive curvature in all directions except the path of reaction. Considering the reactions:

$$A_1 + B + C + \ldots \xrightarrow{k_1} P_1$$

$$A_2 + B + C + \ldots \xrightarrow{k_2} P_2$$

where, A_1 is the lighter isotopic molecule, A_2 the heavier and P_1 and P_2 are the products, the rate constants k_1 and k_2 are given by Eyring's (52) method:

$$k_1 = K_1\left(\frac{C_1^{\ddagger}}{C_{A_1}C_B\ldots}\right)\left(\frac{kT}{2\pi m_1^*}\right)^{1/2} \cdot \frac{1}{\delta_1} \qquad (1)$$

$$k_2 = K_2\left(\frac{C_2^{\ddagger}}{C_{A_2}C_B\ldots}\right)\left(\frac{kT}{2\pi m_2^*}\right)^{1/2} \cdot \frac{1}{\delta_2} \qquad (2)$$

where,

K_1 is the transmission coefficient;

$C^{\ddagger}$ is the concentration of the activated complex;

m^* is the effective mass of the complex along the co-ordinate of decomposition; (it is usually considered as the reduced mass of the atoms whose bond is actually involved in the reaction).

δ is the length of the top of the potential barrier which the complex traverses.

The contribution to the rate constants by tunneling through the barrier have been neglected in equations (1) and (2), but a correction for this can be applied as shown by Bigeleisen (51). To a high degree of approximation the potential energy surfaces for isotopic molecules are identical i.e. $\delta_1 = \delta_2$ and from equations (1) and (2):

$$\frac{k_1}{k_2} = \frac{K_1}{K_2} \times \frac{C_1^{\ddagger}}{C_2^{\ddagger}} \times \frac{C_{A_2}}{C_{A_1}} \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \qquad (3)$$

Replacing the ratio of the concentration of individual molecules by the ratio of the complete partition functions Q's we get from equation (3):

$$\frac{k_1}{k_2} = \frac{K_1}{K_2} \times \frac{Q_1^{\ddagger}}{Q_2^{\ddagger}} \times \frac{Q_{A_2}}{Q_{A_1}} \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \qquad (4)$$

The zero of the potential energy curve of the normal molecule is taken as the zero of the energy scale of the normal molecule, while the zero in the saddle of the potential energy surface of activated complex is chosen as a zero for the evaluation of ratio of the partition functions of the isotopic activated complex.

In view of Bigeleisen and Mayer's (54) treatment the ratio of the complete partition functions of two isotopic molecules can be expressed in terms of a function

of the energy levels of two molecules whose rotation is classical at the reaction temperature. Hence equation (4) can be expressed as:

$$\frac{k_1}{k_2} = \frac{K_1}{K_2} \times \frac{f}{f^{\ddagger}} \left(\frac{m_2^*}{m_1^*} \right)^{1/2} \tag{5}$$

where,

$$f = \frac{Q_{A_2}}{Q_{A_1}} \cdot \Pi_i \left(\frac{M_{i(1)}}{M_{i(2)}} \right)^{3/2} \tag{6}$$

M_i's are the masses of the isotopic atoms in the isotopic molecules.

But from the theory of equilibrium isotope effect the ratio of the partition functions can be expressed as:

$$\frac{Q_{A_2}}{Q_{A_1}} = \frac{S_1}{S_2} \left(1 + \sum_i^{3n-6} G(u_i)\Delta u_i\right) \tag{7}$$

$$\frac{Q_2^{\ddagger}}{Q_1^{\ddagger}} = \frac{S_1^{\ddagger}}{S_2^{\ddagger}} \left(1 + \sum_i^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger}\right) \tag{8}$$

where, 'S' is the symmetry number to account for the identity of nuclei, $G(u_i) = \frac{1}{2} - \frac{1}{u_i} + \frac{1}{(e^{u_i}-1)}$, $u_i = \frac{h\nu_i}{kT}$ and $\Delta u_i = \frac{h}{kT}(\nu_{i(1)} - \nu_{i(2)})$. Using equations (6), (7) and (8), equation (5) is rearranged to

give the following in case of Δu_i small:

$$\ln \frac{k_1}{k_2} \cdot \frac{S_2}{S_1} \cdot \frac{S_1^{\ddagger}}{S_2^{\ddagger}} = \ln \frac{K_1}{K_2} + \frac{1}{2} \ln \frac{m_2^*}{m_1^*} + \sum_i^{3n-6} G(u_i)\Delta u_i - \sum_i^{3n'-6} G(u_i)^{\ddagger} \Delta u_i^{\ddagger} \qquad (9)$$

Expanding equation (9) and neglecting higher terms in the expansion we get:

$$\frac{k_1}{k_2} = \frac{K_1}{K_2} \cdot \frac{S_1}{S_2} \cdot \frac{S_2^{\ddagger}}{S_1^{\ddagger}} \left(\frac{m_2^*}{m_1^*} \right)^{1/2} \left(1 + \sum_i^{3n-6} G(u_i)\Delta u_i - \sum_i^{3n'-6} G(u_i^{\ddagger}) \Delta u_i^{\ddagger} \right) \qquad (10)$$

For systems above room temperature having a distribution in velocities of the reacting isotopic molecules, it has been found by Hirschfelder and Wigner (55) that the transmission coefficient ratio is very nearly equal to unity. Since m_2^*/m_1^* term in equation (10) is in general larger than unity, the lighter molecules have the greater rate constant. This is true also in view of the fact that usually the reacting molecule is bound more tightly than the activated complex. If however, the activated complex is bound very tightly such that:

$$\sum_i^{3n-6} G(u_i)\Delta u_i > \sum_i^{3n'-6} G(u_i^{\ddagger}) \Delta u_i^{\ddagger}$$

Then the rate constant for the heavier molecule will be greater only when:

$$\sum_{i}^{3n'-6} G(u_i^{\ddagger})\Delta u_i^{\ddagger} > \sum_{i}^{3n-6} G(u_i)\Delta u_i + \frac{1}{2} \ln \left(\frac{m_2^*}{m_1^*}\right) .$$

This will give what is termed as "inverse isotope effect".

Bigeleisen and Wolfsberg (56) has further pointed out that in view of the breaking of molecules into two fragments, the reduced masses of the fragments should be used instead of the reduced masses of the activated complex along the reaction co-ordinate. This has been discussed in more detail by Wolfsberg (57). He has considered a molecule X-A-B-Y, where A-B bond is broken. The reduced mass term will be nearly AB/A+B for the case where the A-B bond is stronger than the X-A and B-Y bonds. If A-B bond is a weak bond then the reduced mass will be:

$$\frac{(m)_{X-A} \cdot (m)_{X-B}}{(m_{X-A}) + (m_{X-B})}$$

This is because the mass centres m_{X-A} and m_{X-B} are separated.

It is not possible to find out the specific value of the rate constant in view of the somewhat unknown nature of the activated complex, but one can fix the following limits:

(i) For activated complex similar to the reactant:

$$\sum_{i}^{3n-6} G(u_i)\Delta u_i \simeq \sum_{i}^{3n'-6} G(u_i)^{\ddagger} \Delta u_i^{\ddagger}$$

and the ratio of rate constants in equation (10) is equal to the ratio of reduced masses. The ratio of course takes its least value.

(ii) For activated complex similar to the product, there is a bond rupture and

$$\sum_{i}^{3n'-6} G(u_i)^{\ddagger} \Delta u_i^{\ddagger} \simeq \left(\sum_{i}^{3n-6} G(u_i)\Delta u_i \right)_{(\text{product})}$$

(iii) For a diatomic molecule we have:

$$\left(\sum_{i}^{3n-6} G(u_i)\Delta u_i \right)_{(\text{product})} = 0$$

and the ratio of rate constants is maximum. In fact the observed effect is usually intermediate between the two cases.

In studying a laboratory reaction, it is necessary to know how the isotopic composition of the products and the remaining reservoir vary in time. In general for a competitive reaction of the type:

$$A_1 + B \xrightarrow{k_1} X_1 + Y$$

$$A_2 + B \xrightarrow{k_2} X_2 + Y$$

where the subscripts 1 and 2 are for the lighter and heavier isotopic species respectively, we can write for the first order reaction:

$$\frac{dX_1}{dt} = k_1(A_{0_1} - X_1)(B)$$

$$\frac{dX_2}{dt} = k_2(A_{0_2} - X_2)(B)$$

But at t = 0, $X_1 = 0$ and $X_2 = 0$. Therefore

$$\frac{k_1}{k_2} = \frac{\ell n(\frac{A_{0_1}}{A_{0_1} - X_1})}{\ell n(\frac{A_{0_2}}{A_{0_2} - X_2})} \qquad (11)$$

If the fraction of molecules that have reacted is given by:

$$f = \frac{X_1 + X_2}{A_{0_1} + A_{0_2}} = \frac{X_1}{A_{0_1}} \times \frac{1 + \frac{X_2}{X_1}}{1 + \frac{A_{0_2}}{A_{0_1}}}$$

and the ratio $r = \frac{X_2/X_1}{A_{0_2}/A_{0_1}}$ where, A_{0_2}/A_{0_1} is the isotopic ratio in the initial reactant and X_2/X_1 is the ratio after the fraction 'f' has reacted, then equation (11) yields:

$$\frac{k_1}{k_2} = \frac{\ell n(1 - f \cdot \frac{1 + A_{O_2}/A_{O_1}}{1 + X_2/X_1})}{\ell n(1 - rf \cdot \frac{1 + A_{O_2}/A_{O_1}}{1 + X_2/X_1})} \tag{12}$$

But the heavier isotopes of elements carbon, oxygen, nitrogen and sulphur are relatively rare i.e. we can take:

$$1 + A_{O_2}/A_{O_1} \simeq 1$$

and also

$$1 + X_2/X_1 \simeq 1 \quad .$$

Therefore equation (12) gives the ratio of rate constants as:

$$\frac{k_1}{k_2} = \frac{\ell n(1-f)}{\ell n(1-rf)} \quad .$$

Nakai and Jensen (50) have developed for a first order reaction an expression for the fractionation factor 'R' which is the ratio of $\frac{X_2}{X_1} / \frac{A_2}{A_1}$ where both X and A refer to the same time 't'. They find,

$$R = \frac{F^{(k_2/k_1)^{-1}} - F}{1 - F}$$

where 'F' is the ratio of the amount of residual reactant at time 't' to initial reactant at time t = 0.

EXPERIMENTAL

Microbiological Reduction of Sulphite

In the microbiological reduction of sulphite ($SO_3^=$) the following organisms were used: *Salmonella paratyphi A* (A.T.C.C. #274), *Salmonella oranienberg* (NCTC # 3746), *Salmonella typhi*, *Salmonella paratyphi C* and *Salmonella heidelberg*. Eighteen hour cells were inoculated to one litre of Trypticase Soy Broth (T.S. broth, Baltimore Biological Co. Ltd.) to which was added 10 ml. of 10% w/v sterile solution of Na_2SO_3 after autoclaving and cooling, giving a final concentration of 0.1% w/v Na_2SO_3. T.S. broth was found to be the best medium, as others tested caused autoreduction of $SO_3^=$ due to reducing sugars present in the medium. It was noted in one case with Bacto Nutrient Broth that the H_2S produced by autoreduction of $SO_3^=$ was enriched in S^{32} by 0.5 per cent.

The apparatus for trapping H_2S for isotopic study is shown in figure IV. Gas was bubbled through the medium to prevent clumping of the organisms and to accomodate flushing of the H_2S from the medium. Two ports were available on the culture flask, the first for the addition of nutrients and the second for the collection of 1 ml. aliquots for plate counts.

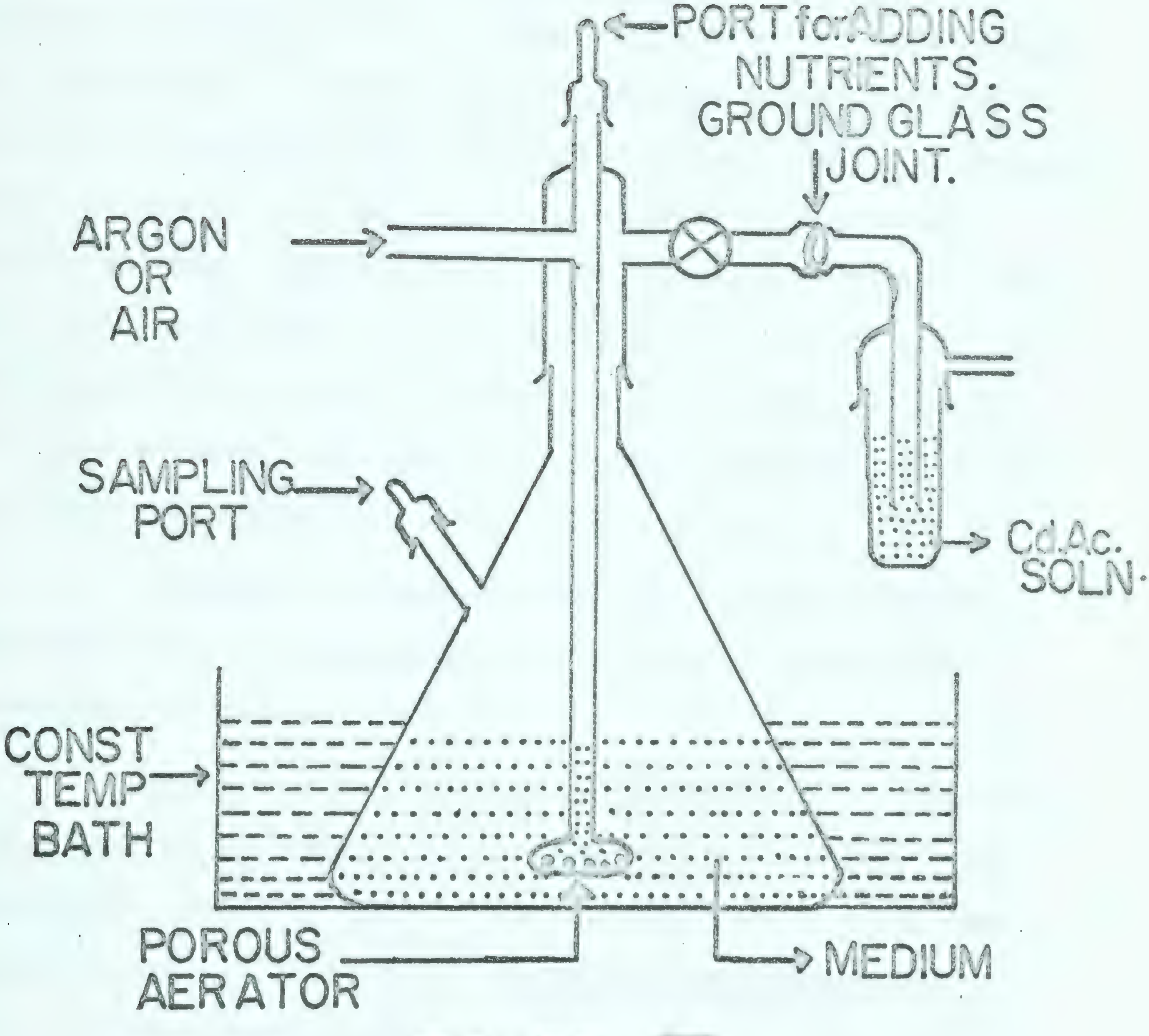

PORT for ADDING NUTRIENTS.
GROUND GLASS JOINT.
ARGON OR AIR
SAMPLING PORT
Cd.Ac. SOLN.
CONST TEMP BATH
POROUS AERATOR
MEDIUM

FIGURE — IV.

The gaseous metabolic end-product, H_2S, was trapped in cadmium acetate solution as CdS. The CdS was allowed to accumulate from 4 to 6 hours and then converted to Ag_2S by the addition of 0.1N $AgNO_3$. In order to obtain Ag_2S in a form easy to filter, the mixture was boiled for one minute to coagulate Ag_2S before filtration. The Ag_2S precipitate was washed with concentrated ammonia to remove any silver chloride (AgCl) present and then washed several times with deionized water. The Ag_2S was then collected on 47 mm millipore filter discs of 0.45 micron pore size, dried at 37°C and weighed. The amount of sulphur released per unit time was calculated from the weights of Ag_2S.

Initially a general study was conducted using various species of Salmonella under aerobic as well as anaerobic conditions of growth.

Later, to realize a higher percentage of sulphite reduction and maintain the cell population throughout the experiment, fixed amounts of sterile glucose solution were added at regular intervals. Salmonella heidelberg was used in these experiments.

One ml. aliquots of culture medium were taken every four hours and logarithmically diluted and 0.1 cc of each dilution was plated in triplicate on T.S. Agar. The plates were incubated at 37°C for 24 hours and then counted using the Quebec Colony counter.

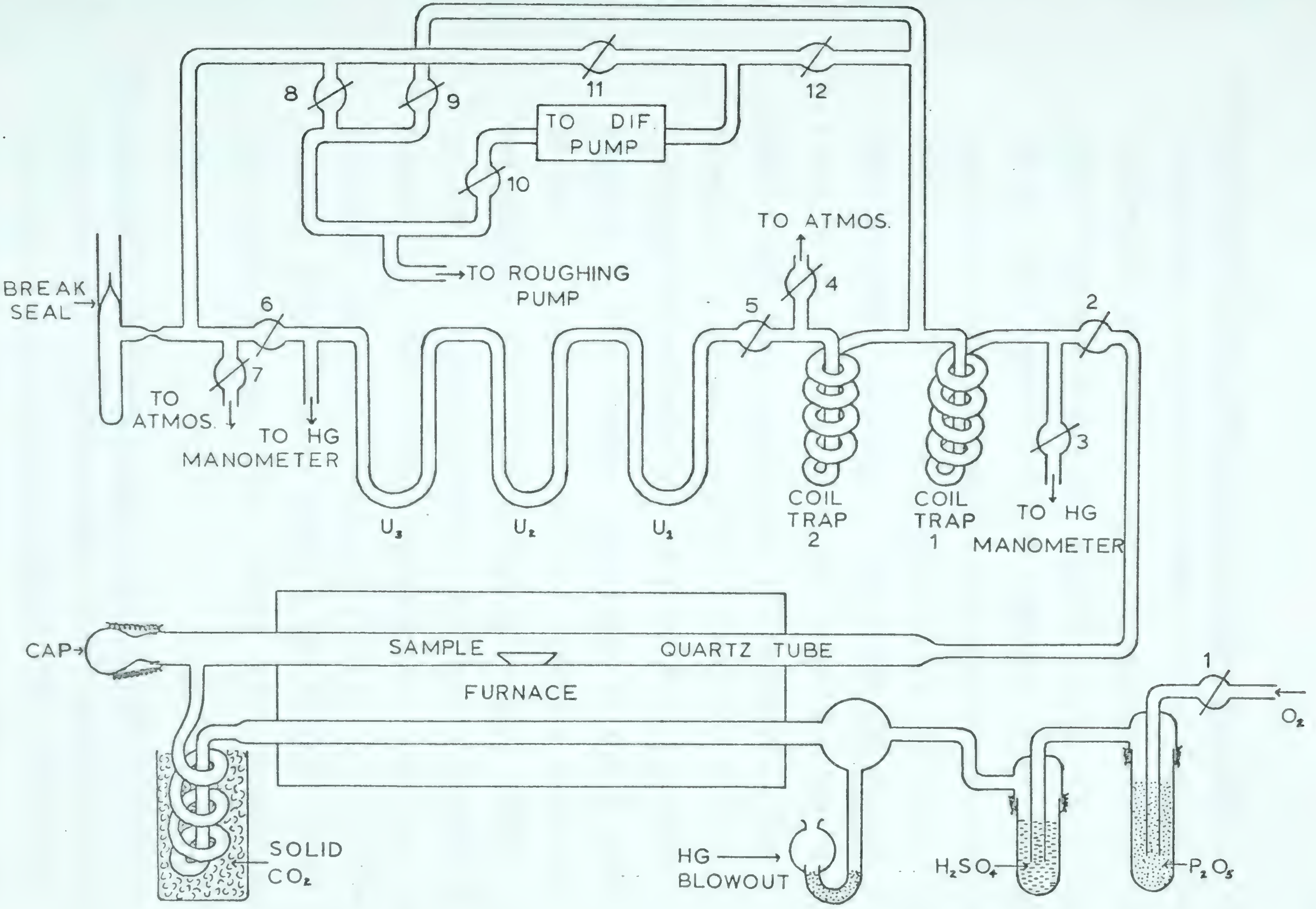
Preparation of Sulphur Dioxide (SO_2) Samples for Mass Spectrometric Analysis

The combustion characteristics of Ag_2S show that it is essential to heat the sample above a temperature of 1000°C in order to decompose any silver sulphate formed. To fulfill this requirement for complete combustion of silver sulphide the prepared samples were burned at 1200°C in a furnace specially built for the purpose. The schematic diagram of the furnace is shown in figure V. Two heating elements each of resistances about 17 ohms were connected in series with a temperature controller Model 2706-644-800 manufactured by Assembly Products Inc., Chesterland, Ohio and used with the 200 volts outlet. A thermocouple signal was sensed by the controller and the temperature was retained constant at 1200°C.

The actual combustion of silver sulphide samples involve the following steps:

(1) Flaming of the sample processing line: The section of the line between the quartz tubes and stopcocks 4, 5, 9 and 12 were flamed while passing oxygen through. With the cap removed from the quartz tube and stopcock 2 closed, all of the sample processing line was flamed under vacuum to remove any gases absorbed in the glass walls.

FIG.-V. FURNACE FOR THE BURNING OF SILVER SULPHIDE SAMPLES



(2) Insertion of the sample in the combustion tube: With stopcocks 2, 4, 9 and 12 closed, stopcock 4 was opened to atmospheric pressure. The cap was replaced on the quartz tube and oxygen was passed through for a time. The two coil traps were then immersed in liquid oxygen, the cap removed, the sample in a quartz boat inserted into the middle of the quartz tube of the furnace and the cap quickly replaced. Combustion was immediate, but five minutes were usually allowed for the burning. The cap was then removed and the stopcocks 2 and 4 closed. Any oxygen liquified within the coil traps was pumped away using the roughing pump by opening stopcock 9 very slowly. (Stopcocks 10 and 12 were closed.) After a short time of roughing stopcock 9 was closed. The liquid oxygen baths were removed from the two coil traps to release any oxygen trapped in the frozen SO_2. The SO_2 was refrozen into one of the coil traps and the sample was again pumped upon with the roughing pump. Then stopcock 9 was closed, 10 and 12 were opened to pump on the sample with the diffusion pump.

(3) Transfer of the sample to U-tubes and purification: To transfer the collected sample to the U-tube, stopcocks 6 and 12 were closed and 5 opened after putting an acetone solid carbon dioxide mixture beneath U_2 and

liquid air beneath U_3. (In the meantime, the liquid oxygen bath was removed from the coil trap after transfer, stopcock 5 was closed, 4 opened, the cap replaced and oxygen passed through the coil traps with flaming.) The acetone solid carbon dioxide mixture was used to remove any water present. This was essential since there exists a possibility of exchange of oxygen between water and SO_2 (Brown and Drury (58)). Hence for a better purification, liquid air was interchanged two or three times between U_1 and U_3. Finally the sample was collected in U_3 and the liquid air bath was replaced by a solid-liquid alcohol bath. At this temperature, the vapour pressure of carbon dioxide is a few mm Hg while that of SO_2 is about 0.001 mm Hg. CO_2 was therefore removed by pumping with the diffusion pump for a short while by opening stopcocks 6 and 11.

(4) Collection of SO_2 sample in break seal: The final stage is the transfer of SO_2 to the break seal for analysis in the mass spectrometer. Care was taken to flame and pump the break seal line before collecting the sample in it. Stopcocks 8 and 11 were closed and the break seal immersed in liquid air. After removing the solid-liquid alcohol bath from U_3, stopcock 6 was opened and the sample frozen in the break seal. The break seal was

then finally removed from the line by collapsing the constriction with a mild flame. While removing the break seal it was kept immersed in liquid air to avoid any loss of the SO_2 sample. In the mean time the combustion line from the furnace to stopcock 5 had been flamed several times with the oxygen flowing and therefore another sample of silver sulphide could be introduced and undergoing combustion while the sample purification line was flamed under vacuum.

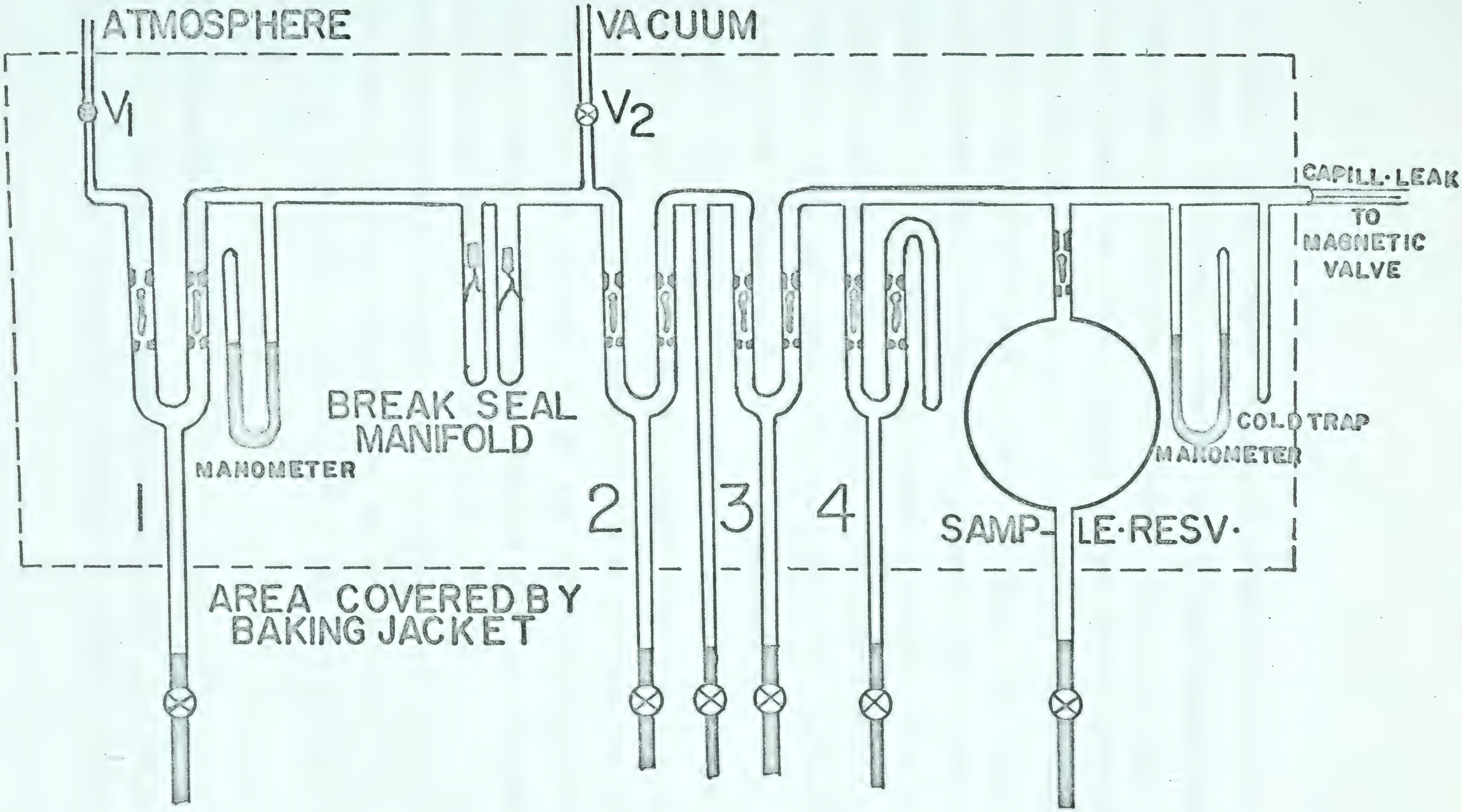
Mass Spectrometry

Sample Handling System

The sample handling system is shown in figure VI. An iron slug was inserted into each break seal tube and the tube was then sealed to the manifold as shown. All of the line is glass with the exception of V_1 and V_2 which are metal Hoke valves. There is a similar handling system for a standard reference SO_2 sample, the difference being that storage bulbs with valves are connected into the manifold system.

To attach the break seals, mercury cutoffs 2 and 3 were closed. V_2 was closed and V_1 was opened slowly to bring the manifold up to atmospheric pressure. A blowing tube was attached to the V_1 outlet and the break seals

FIG-VI·SAMPLE HANDLING SYSTEM ON MASS·SPEC·



attached. V_1 was closed, V_2 opened slowly and the line evacuated. The mercury cutoffs 2 and 3 were lowered and the whole line gently flamed under vacuum. V_2 and mercury cutoff 3 were then closed, liquid air was placed under the break seal tube to be opened. The iron slug was lifted up with a magnet and dropped to break the seal. The liquid air trap was then removed and the pressure read by the manometer. The mercury level between cutoffs 3 and 4 was adjusted appropriately, the cutoff 2 was closed and 3 opened to admit the sample into the reservoir. The mercury level in the sample reservoir was adjusted to give the required ion current in the mass spectrometer. Mercury cutoff 4 can be used to store a sample to be run at a latter time. In this study, a sample of SO_2 of the same isotopic composition was analyzed, stored and then analyzed again after a number of SO_2 samples prepared from the bacterial reductions were analyzed. This checked the drift of the mass spectrometer in time. The capillary leaks from both the sample and the standard lines led to magnetic valve system as described by McKinney et al (59) and Wanless and Thode (60).

The Mass Spectrometer

The mass spectrometer used for this investigation was basically a 12" radius - 90° magnetic analyser which

separated ion currents of different masses. The sulphur dioxide was ionized by a stabilized electron beam moving at 90° to the gas flow direction. The ions formed due to electron bombardment were then accelerated by a potential of the order of 3.5 KV and subsequently separated in the magnetic field. A wide slit and a narrow slit were used in the ion collector assembly for the collection of ion currents of masses 64 and 66 respectively.

During the first part of the study more conventional circuitry was used in simultaneous collection. A portion of the voltage developed by the larger ion current was fed back inversely by a calibrated 5-figure potentiometer to cancel the voltage developed by the smaller ion current. This null was detected on a center zeroed pen recorder. The reading of potentiometer for the null condition was recorded in turn for the standard and the unknown sample.

During the latter part of the study, digital equipment was used in the simultaneous collection circuitry. This consisted of three parts, Model DY-2401A integrating digital voltmeter, a Dymec Model DY-2211B voltage-to-frequency converter and a Hewlett-Packard J66-562A digital recorder. The basic principle of operation of the digital equipment is that the input voltage is integrated over a

selected time by the integrating digital voltmeter and subsequently converted to a proportional frequency. This frequency is referred to a standard time base frequency while passing through a counter section. The printed or visually displayed voltage is then essentially the ratio of the converted frequency to that of the standard time base. If instead of this time base reference frequency in denominator, an additional voltage to frequency converter is used, the ratio of two voltages can be displayed or printed. Ten ratios 'R' were printed alternately for the standard and the unknown samples. Six such sets for each were printed and δ^{34} (uncorrected) determined as:

$$\delta^{34}(\text{uncorrected}) = \left(\frac{R(\text{sample})}{R(\text{Sulphite})} - 1\right) \times 1000$$

The precision with-in an analysis and the accuracy of S^{34}/S^{32} comparisons on repeated samples was generally better than 0.02 per cent (standard deviation). The δ-value was corrected because of oxygen isotopes (mass 66, $S^{34}O^{16}O^{16}$, $S^{32}O^{16}O^{18}$) by multiplying by a correction factor of 1.090. (Hulston and Shilton (61)). This corrected value is

$$\delta^{34} = \left(\frac{S^{34}/S^{32}(\text{sample})}{S^{34}/S^{32}(\text{sulphite})} - 1\right) \times 1000 \quad .$$

RESULTS AND DISCUSSION

General Survey

The results of the general survey are summarized in Table I and Figure VII.

It was found that the product H_2S produced during 24 hours was depleted in S^{34} by as much as 3.35 per cent (33.5 δ-units) in comparison with the isotopic composition of the reactant sulphite.

All of the species reported effected alterations of the S^{34}/S^{32} ratio of the same order although the fractionation realized for anaerobic and aerobic conditions varied considerably with the species. The extreme case was with paratyphi A where anaerobically the δ^{34} depletion was 33.5 δ-units while aerobically, it was 20.7 δ-units.

In most cases, the anaerobic reductions realized larger isotope fractionations than the aerobic cases. Anaerobically, the amount of H_2S produced was about 3 times greater than in the aerobic cases. The percentage reduction never exceeded 15 per cent. Salmonella heidelberg showed the smallest difference in isotope fractionation between the aerobic and anaerobic cases, but it is seen that replicate runs at different times lack in reproducibility and in one

FIGURE VII S^{34}/S^{32} FRACTIONATION IN SULPHITE REDUCTION BY SALMONELLA SP. (Aerobic-Anaerobic Comparisons, Initial Conditions Unaltered)

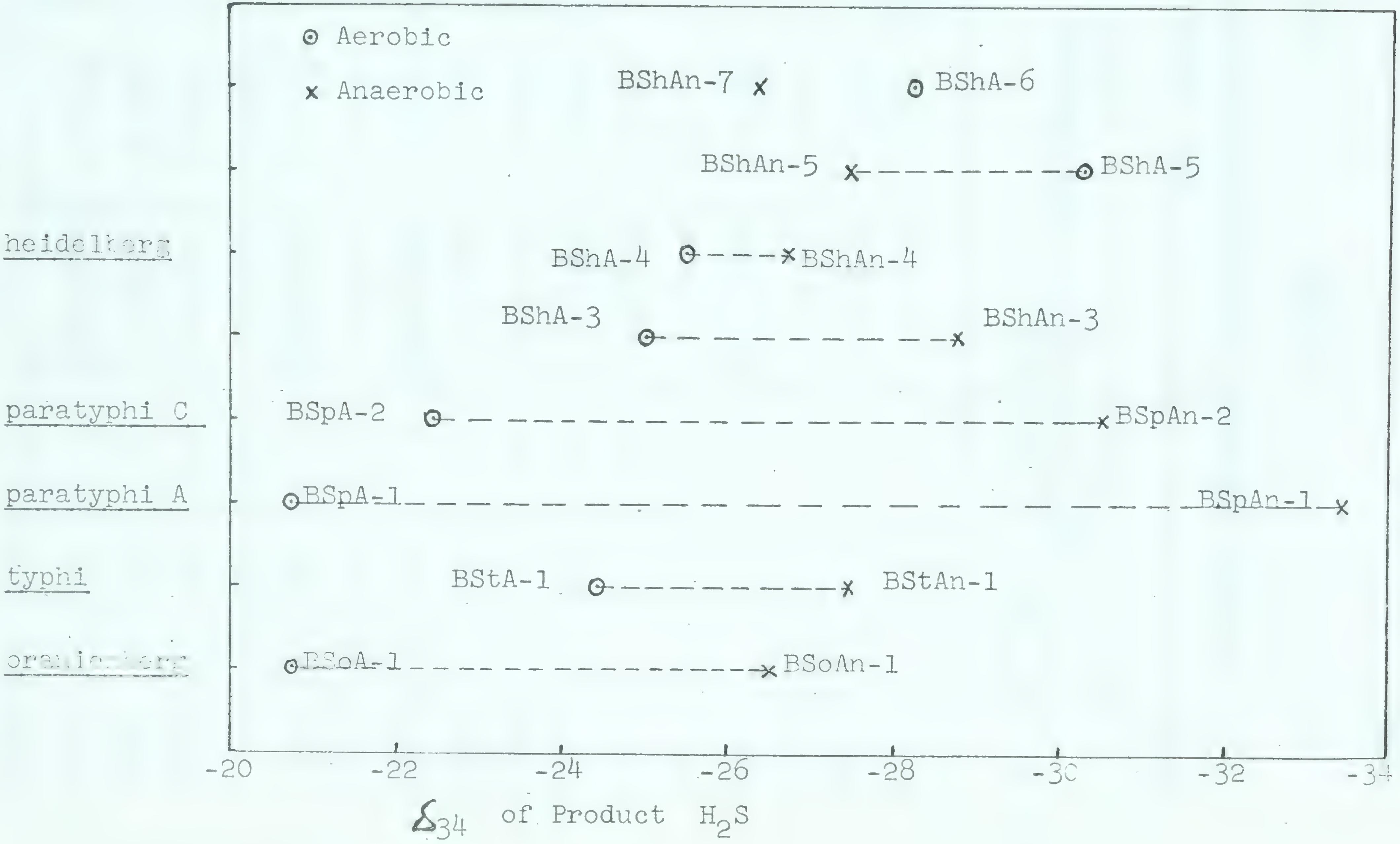


TABLE I

SULPHITE REDUCTION WITH SALMONELLA SPECIES

AEROBIC-ANAEROBIC COMPARISONS (Initial conditions unaltered)

Species	Designation	Condition of Growth	Temperature °C	Reduction Time In Hours	δ^{34} of H_2S
oranienberg	BSoA-1	Aerobic	37	24	-20.7
oranienberg	BSoAn-1	Anaerobic	37	24	-26.5
typhi	BStA-1	Aerobic	37	24	-24.4
typhi	BStAn-1	Anaerobic	37	24	-27.5
paratyphi A	BSpA-1	Aerobic	37	48	-20.7
paratyphi A	BSpAn-1	Anaerobic	37	48	-33.5
paratyphi C	BSpA-2	Aerobic	37	24	-22.5
paratyphi C	BSpAn-2	Anaerobic	37	24	-30.5
heidelberg	BShA-3	Aerobic	37	24	-25.1
heidelberg	BShAn-3	Anaerobic	37	24	-28.8
heidelberg	BShA-4	Aerobic	37	24	-25.5
heidelberg	BShAn-4	Anaerobic	37	24	-26.7
heidelberg	BShA-5	Aerobic	37	24	-30.3
heidelberg	BShAn-5	Anaerobic	37	24	-27.5
heidelberg	BShA-6	Aerobic	25	24	-28.2
heidelberg	BShAn-7	Anaerobic	37	24	-26.4

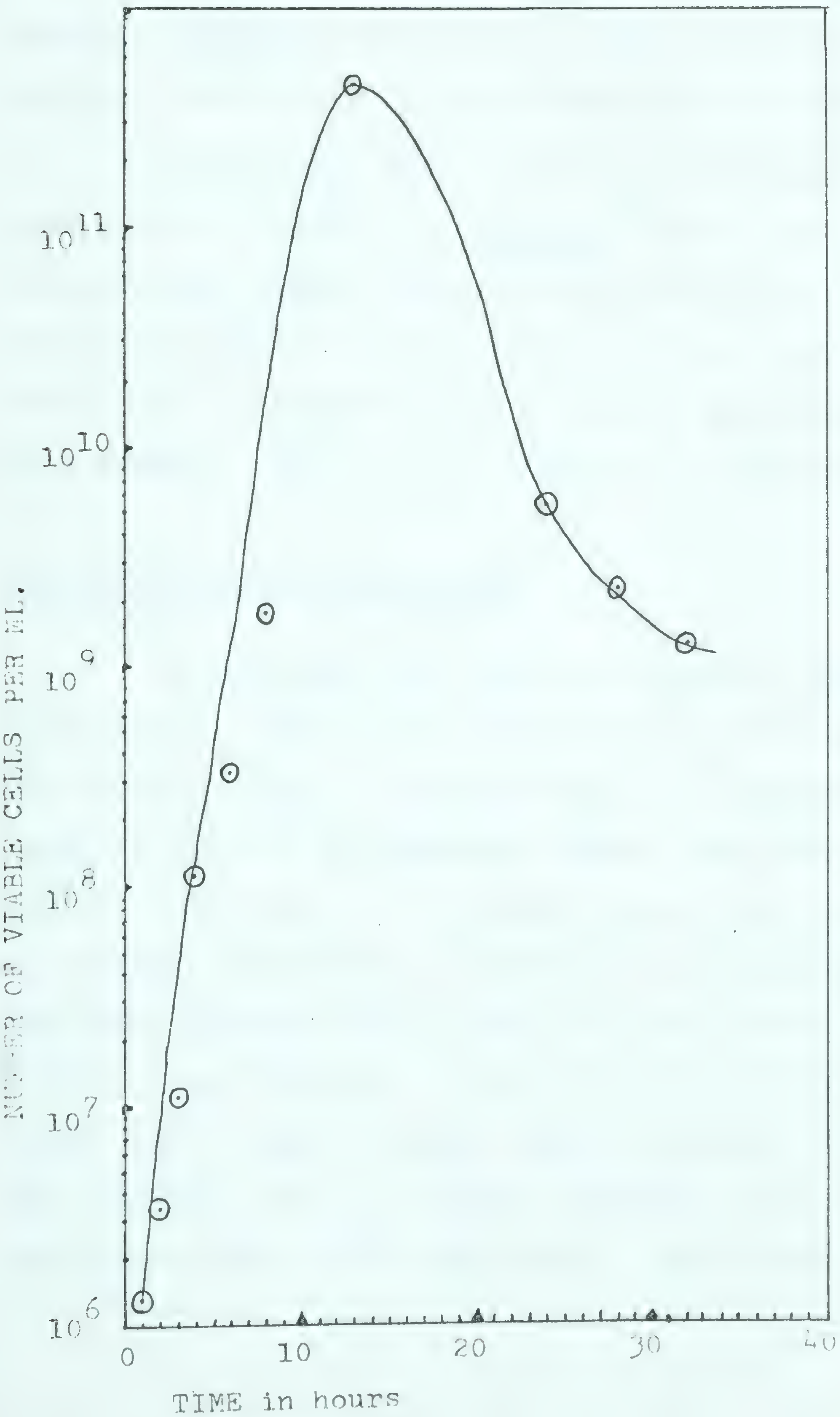
case, the larger fractionation was found aerobically.

The findings of Harrison and Thode et al (46), and Kaplan and Rittenberg (48, 49) indicate that the isotopic fractionation varies inversely as the rate of reduction. The results of the general survey would disagree with these findings if the H_2S production rate were constant over the whole 24 hours.

Qualitative observations however indicate that the H_2S production was not constant over the time interval. Production did not commence until about 8 hours after inoculation and was decreasing noticeably towards the end of 24 hours.

In an attempt to investigate the conditions further, counts were made of the bacteria every four hours with the initial conditions unaltered. Figure VIII shows how the number of viable cells varied in time. A peculiarity exists in that the cell population rapidly climbs and reaches a maximum at about 13 hours. It then rapidly decreases stabilizing at less than 1 percent of the maximum value. The same effect existed anaerobically and aerobically but in the latter cases the counts were about 50 times higher. This phenomenon is still being investigated in the department of microbiology and no explanation is offered for it here other than that it appears to be a poisoning effect.

FIGURE VIII POPULATION DETERMINATION (Initial Conditions Unaltered)



It is quite clear that the production rate per cell must be varying considerably under these conditions and it is therefore impossible to derive any relationship between the reduction rate and the isotope fractionation study.

The general survey however is informative in showing that a number of Salmonella species are capable of fractionating sulphur isotopes during reduction of sulphite. Further, the maximum effect ($\delta = -33.5$) realized is significantly high in comparison to the results generally found by other workers. This will be discussed in more detail later.

Continuous Feeding Experiments

It was found that regular feeding of glucose could realize a more stable H_2S production rate and further, the reduction could be carried nearly to completion. Initially plots of H_2S produced versus time gave reasonably straight lines which would indicate zero order reaction. The isotope fractionation however is not consistent with zero order kinetics and so the yield was plotted in terms of first order kinetics. Plots of $\ell n((SO_3^=)_0/(SO_3^=)_t)$ versus time 't' gave straight lines consistent with first order kinetics (50). It appears therefore that at the rates encountered in the experiment, conventional ways of

determining the reaction order are not sufficiently sensitive whereas the dependence of isotope fractionation on percentage reaction clearly determines the order. It should be noted however that whereas first order kinetics are observed in any one reduction, intercomparisons with different concentrations recently made do not find that the rate is proportional to the concentration. This is the fundamental definition of first order kinetics and this anomaly must be due to the poisoning effect previously noted. The decrease of production rate with concentration has also been noted by Harrison and Thode (46) and Kaplan and Rittenberg (49) in the reduction of sulphate by Desulphovibrio desulphuricans.

The results of three continuous feeding experiments are shown in Tables II, III and IV and Figures IX, X and XI. In Figure XII counts are shown under aerobic and anaerobic conditions.

The counting results indicate that the population also goes through a maximum in the continuous feeding experiments. This maximum again exists aerobically and anaerobically. The stabilized population in the aerobic case is likewise about fifty times higher than anaerobically. It is seen however that only a small fraction of the H_2S is formed while the population is fluctuating. Therefore,

the rate per unit cell obeys the same kinetics as that of the total rate.

Many ways exist of evaluating the isotope fractionation. In equation (12) on page 22 the factor 'r' refers to the integrated isotopic composition of the product whereas experimentally, the isotopic composition of fractions was measured. Therefore in Figures IX-(a), X-(a) and XI-(a) the integrated isotopic composition is plotted as a function of percentage reaction. The best k_1/k_2 theoretical curves are plotted through the experimental points. The calculated values are given in Tables II, III and IV.

In Figure IX, with the exception of the last determination at 94 hours, the rate fits first order kinetics. The isotope fractionation plot in Figure IX-(a) also shows this sample to be anomalous. This effect is not noted in the other series, but they have not been carried as close to completion. This terminal fraction is interesting in that it has a δ-value of over +62 as compared to the δ-values of -28 near the beginning of the reaction indicating that two fractions whose S^{34}/S^{32} ratio differed by 9 per cent were obtained from this reduction. This is to be expected since the reservoir of unreduced sulphite becomes increasingly enriched in S^{34} because the $S^{32}O_3^{=}$ species are reduced faster. The isotopic composition of the differential

FIGURE IX CONTINUOUS REDUCTION OF SULPHITE BY S.heidelberg
(BShAn-8 series, 37° C, 150 g glucose q.hr., anaerobic)
(△-k_1/k_2; ⊙-ln $(SO_3^=)_o/(SO_3^=)_t$; ⊡-δ_{34} of fraction)

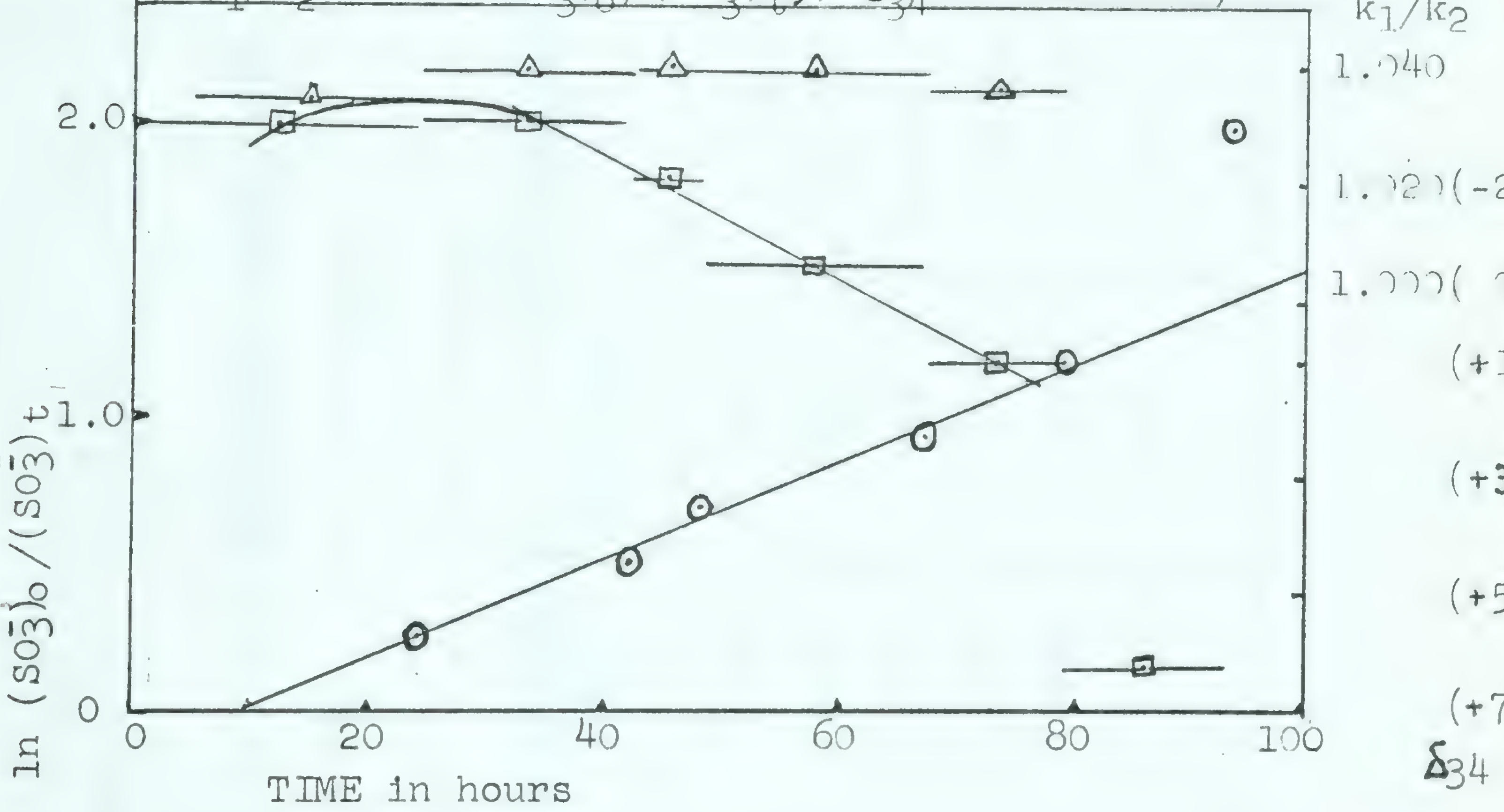


FIGURE IXa INTEGRATED δ_{34} VS. % REACTION

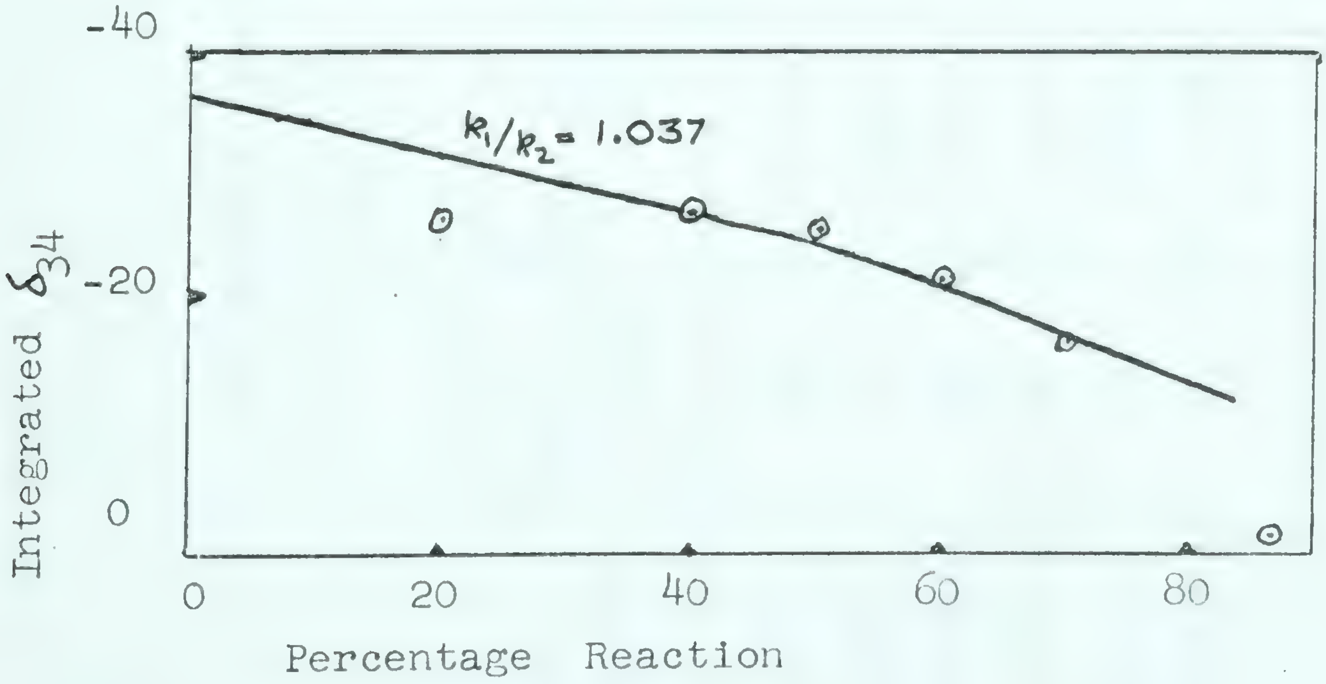


TABLE II.

CONTINUOUS REDUCTION OF SULPHITE WITH *Salmonella heidelberg*

(at 37°C, 150 μg. glucose q. 6 hr. anaerobic)

Sample Designation	Time (t) in hours	Per cent Reaction	$\frac{(SO_3^{=})_0}{(SO_3^{=})_t}$	$\ell n(\frac{(SO_3^{=})_0}{(SO_3^{=})_t})$	δ^{34} of H_2S fractions	Integrated δ^{34}	Calculated ratio k_1/k_2
BShAn-8	24	20.04	1.250	0.2231	-26.6	-26.59	1.032
BShAn-8(a)	42	40.51	1.666	0.5105	-28.2	-27.55	1.037
BShAn-8(b)	48	49.85	1.960	0.6729	-19.4	-26.10	1.038
BShAn-8(c)	67	61.82	2.500	0.9163	- 5.1	-22.25	1.038
BshAn-8(d)	79	73.04	3.333	1.2038	+11.4	-17.43	1.035
BShAn-8(e)	93	91.56	7.353	1.9952	+62.5	- 2.21	1.008

fraction at 100 per cent reduction is theoretically infinitely enriched in S^{34}. The δ-value of the integrated product however approaches zero at 100 per cent reaction since the isotopic composition of the product is then the same as that of the original sulphite.

It is noted that in the initial fraction, the isotope fractionation is low because of the instability of the cell population. This means that the calculated integrated values are also low and hence the best fitting k_1/k_2 value curve is low. This descrepancy can be rectified to some extent by considering each fraction in terms of the isotopic composition of the $SO_3^=$ at the beginning of the fraction i.e. each fraction is considered as a separate reduction. k_1/k_2 values have been accordingly calculated and appear in the last column of Tables II, III and IV.

The conditions of the series in Figure X was initially the same as the series in Figure IX. Again, the isotope fractionation is low near the beginning because of the instability of the cell population. At 40 hours, however, some unknown phenomenon occurred and the reduction rate lowered considerably. Consistent with this, it is seen that the isotope fractionation immediately increased corresponding to k_1/k_2 increasing from 1.034 to 1.041. Thus, the inverse dependence of isotope fractionation on reduction

FIGURE X: CONTINUOUS REDUCTION OF SULPHITE BY S.heidelberg
(BShAn-1 series, 37°C, 150 µg. q. 6 hr., anaerobic)
(△-k_1/k_2, ⊙-ln $(SO_3^=)_0/(SO_3^=)_t$, ⊡-δ_{34} of H_2S fraction)

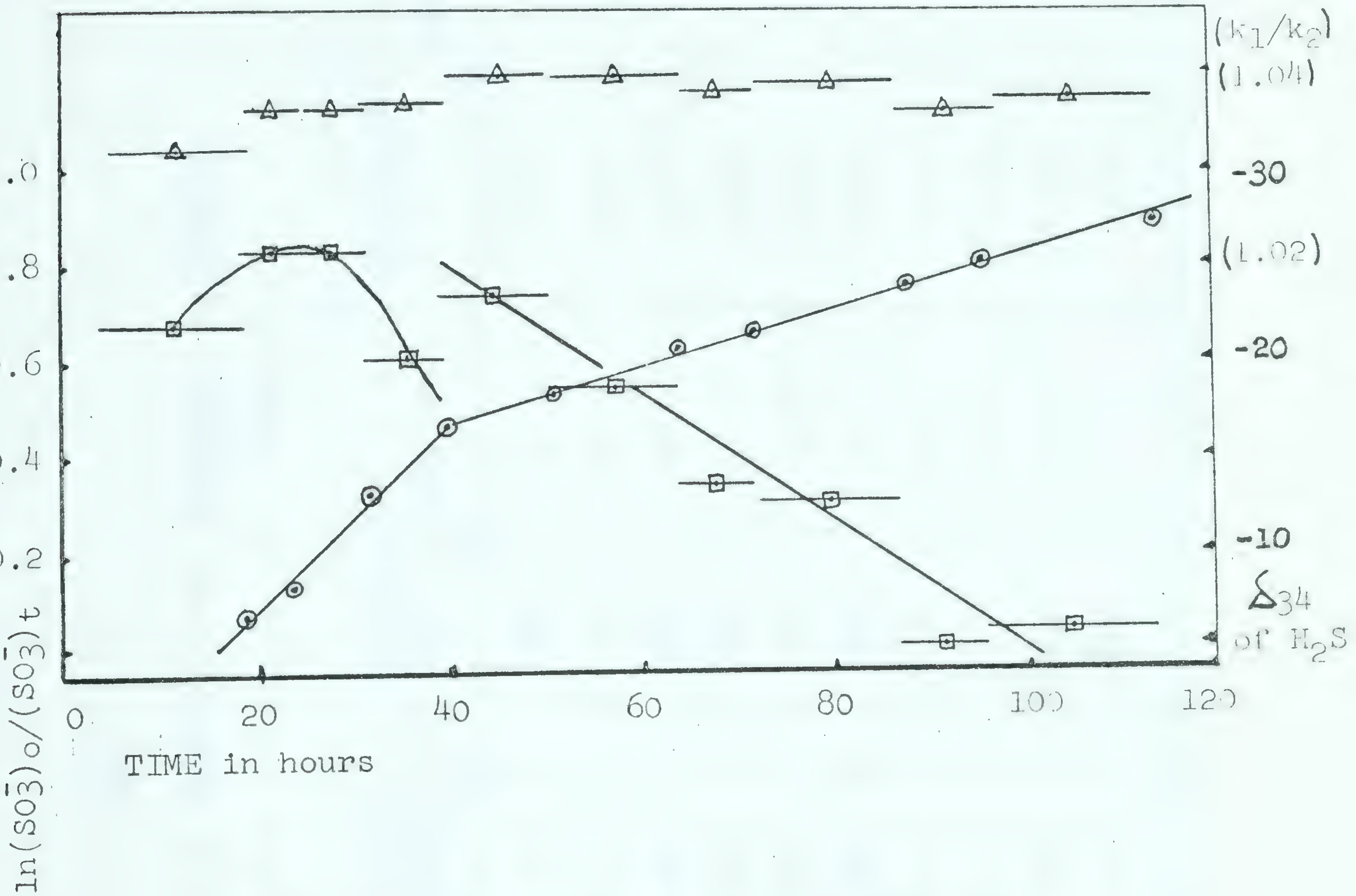


FIGURE Xa INTEGRATED δ_{34} VS. % REACTION

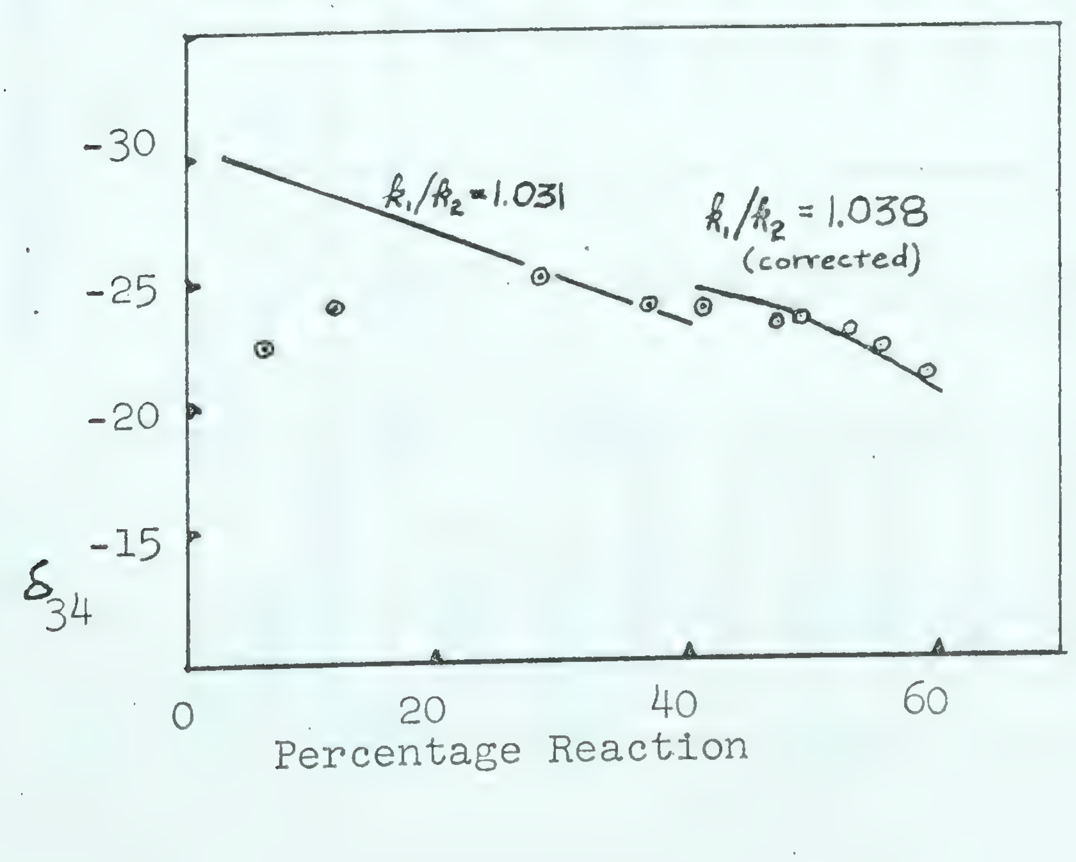


TABLE III.

CONTINUOUS REDUCTION OF SULPHITE WITH *Salmonella heidelberg*

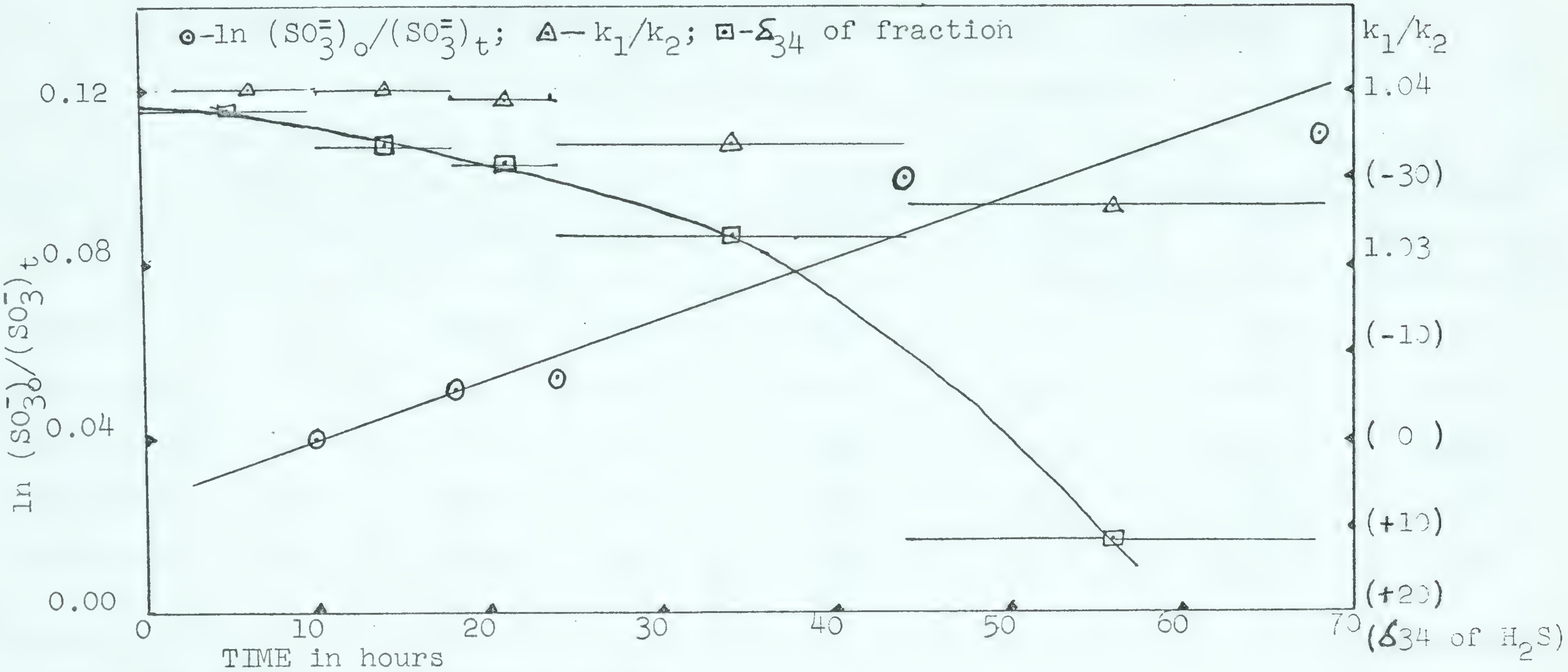
(at 37°C, 150 µg. glocose q. 6 hr. anaerobic)

Sample Designation	Time (t) in hours	Per cent Reaction	$\frac{(SO_3^{=})_0}{(SO_3^{=})_t}$	$\ell n(\frac{(SO_3^{=})_0}{(SO_3^{=})_t})$	δ^{34} of H_2S fractions	Integrated δ^{34}	Calculated Ratio k_1/k_2
BShAn-1	19	6.61	1.070	0.0677	-22.2	-22.20	1.024
BShAn-1(a)	24	12.22	1.139	0.1301	-26.0	-23.94	1.033
BShAn-1(b)	32	28.25	1.392	0.3308	-26.0	-25.09	1.033
BShAn-1(c)	40	37.29	1.592	0.4650	-20.4	-23.95	1.034
BShAn-1(d)	51	41.13	1.698	0.5297	-23.6	-23.92	1.041
BShAn-1(e)	64	46.92	1.883	0.6329	-18.7	-23.27	1.040
BShAn-1(f)	72	48.52	1.942	0.6637	-13.5	-24.02	1.036
BShAn-1(g)	88	53.52	2.141	0.7613	-12.6	-22.99	1.039
BShAn-1(h)	96	55.47	2.245	0.8087	- 4.9	-22.28	1.032
BShAn-1(i)	114	58.87	2.431	0.8883	- 6.0	-21.33	1.036

rate as found by workers with other microorganisms is shown conclusively with Salmonella in this experiment.

The series plotted in Figures XI and XI-(a) differ from the other two series only in that it was performed aerobically. The reduction rate was much slower and the k_1/k_2 ratio was reasonably high (1.040) near the beginning of the reaction. Further, the depletion in S^{34} was a maximum in the first fraction and did not maximize around 25 per cent reaction as in the anaerobic series. The reaction order plot is also peculiar in that it reaches zero at negative times. This indicates that the reduction rate near the beginning was much faster and then stabilized to approximate first order kinetics. The isotopic correlation however is not as good in this trial and on the assumption of first order kinetics, k_1/k_2 steadily decreases in time. It is difficult to evaluate this series and compare it with the others. It is obvious from these graphs and the population determinations that molecular oxygen can also be utilized by Salmonella and this competition significantly lowers the H_2S production rate despite the factor of 50 increase in cell population. In aerobic experiments, it would seem that the oxygen aeration is an additional variable which must be specified if the quantity "reduction rate per unit cell" is to be meaningful.

FIGURE XI CONTINUOUS SULPHITE REDUCTION BY S.heidelberg
(BShA-7 series, 37°C, 150 µg. glucose q.6 hr., aerobic)



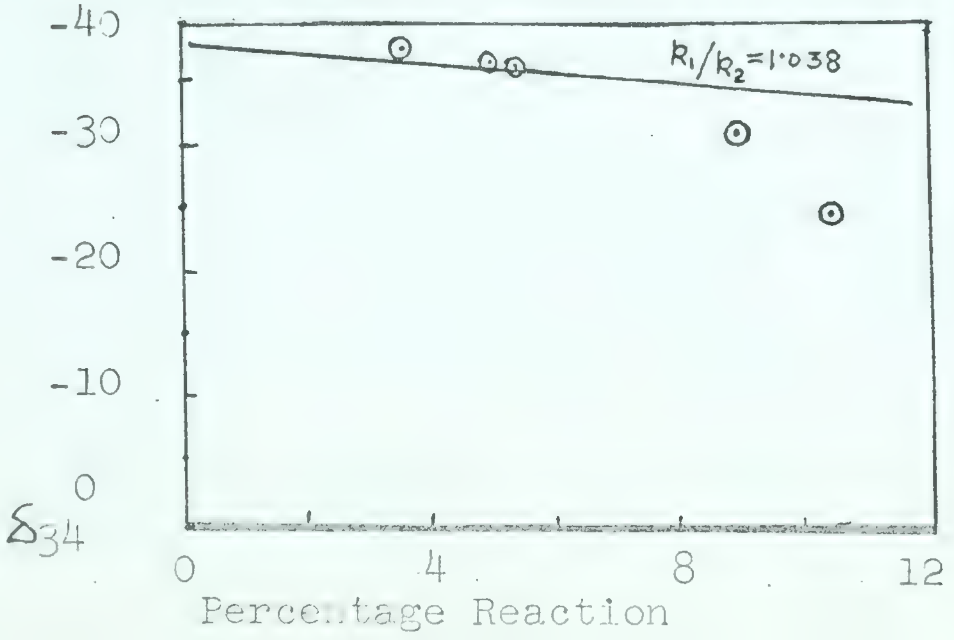


FIGURE XIa
INTEGRATED δ_{34} VS. % REACTION

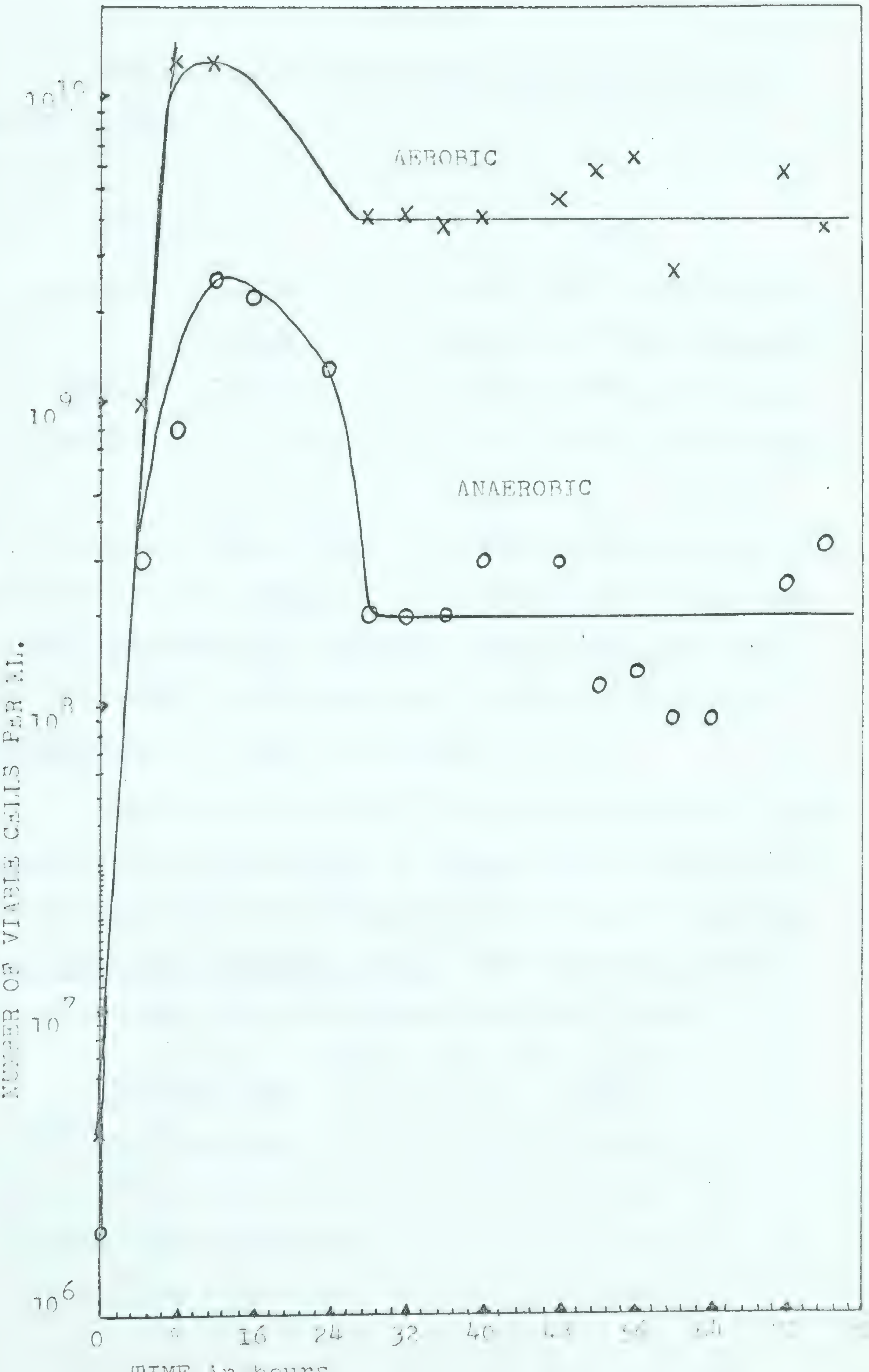
TABLE IV.

CONTINUOUS REDUCTION OF SULPHITE WITH *Salmonella heidelberg*

(at 37°C, 150 μg. glucose q. 6 hr. aerobic)

Sample Designation	Time (t) in hours	Per cent Reaction	$\frac{(SO_3^=)_0}{(SO_3^=)_t}$	$\ell n(\frac{(SO_3^=)_0}{(SO_3^=)_t})$	δ^{34} of H_2S fractions	Integrated δ^{34}	Calculated ratio k_1/k_2
BShA-7	10	3.61	1.038	0.0382	-37.6	-37.61	1.040
BShA-7(a)	18	4.91	1.052	0.0507	-33.5	-36.67	1.040
BShA-7(b)	24	5.31	1.056	0.0535	-30.3	-36.19	1.039
BShA-7(c)	44	8.97	1.098	0.0944	-23.2	-30.82	1.034
BShA-7(d)	68	10.42	1.116	0.1096	+11.8	-24.90	1.027

FIGURE XII POPULATION DETERMINATIONS (CONTINUOUS FEEDING)



The average rates of reduction per unit cell were as follows:

Series		Rate
BShAn-1	Rate I	1.14×10^{-13}mg $S^{=}$/cell/hr.
	Rate II	4.22×10^{-14}mg $S^{=}$/cell/hr.
BShAn-8		1.21×10^{-13}mg $S^{=}$/cell/hr.
BShA-7		1.31×10^{-16}mg $S^{=}$/cell/hr.

For convenience, rates I and II of BShAn-1 assume that the population did not change in the discontinuity. This has not been experimentally verified. These rates are much lower than those usually realized by workers with other microorganisms in reduction studies.

Despite the fact that the mechanisms may be quite different, it is interesting to compare these results with those of Kaplan and Rittenberg (49) for sulphate reduction by Desulphovibrio desulphuricans. The following average k_1/k_2 values are noted for various reduction rates.

Reduction Rate	k_1/k_2
10^{-11}mg $S^{=}$/cell/hr.	1.016
5×10^{-12}mg $S^{=}$/cell/hr.	1.020
2×10^{-12}mg $S^{=}$/cell/hr.	1.030
1×10^{-12}mg $S^{=}$/cell/hr.	1.040

These fractionations and reduction rates were effected by a variety of experimental conditions. The results with *Salmonella* considered in this thesis are comparable with these results.

As discussed on page 10, Harrison and Thode attempted to explain the isotope fractionation in the reduction of $SO_4^=$ by *Desulphovibrio desulphuricans* in terms of the underlying physiological events. According to their theory, the maximum realizable k_1/k_2 is equivalent to that of the inorganic chemical reduction. This assumes that the isotope effect occurs only in the S-O bond breakage. The work of Kaplan and Rittenberg (48, 49) however realizes isotopic fractionations in excess of this theoretical limit.

Further if the S-O bond breakage result were the maximum fractionation permissible in sulphite reduction, the value of k_1/k_2 should not exceed 1.025. In sulphite reduction by *Desulphovibrio desulphuricans*, a k_1/k_2 value of only 1.014 has been reported (48). On the other hand, reductions of sulphite by *Saccharomyces cerevisiae* have realized k_1/k_2 values as high as 1.041 (48) which is the same as the maximum realized with *Salmonella* in this study.

Considerable discussion has been given to k_1/k_2 values of such magnitude (48). Possible explanations include a combination of two or more kinetic isotope effects where a pool of intermediate is built up and the possibility of near equilibrium conditions existing at some step in the mechanism (Equilibrium isotope effects are generally higher than kinetic).

One can presumably place an upper limit on the expected isotope effect on the basis of equation (10) developed in the theory. If the maximum limit is considered, then $G(u_i)\Delta u_i$ of the reactant refers to the sulphite ion and $G(u_i^{\neq})\Delta u_i^{\neq}$ is zero (implying complete dissociation of the products). In equation (10) the symmetry numbers 'S' cancel as the molecules being considered contain only one atom of the element for which an exchange is being made. The equation simplifies to:

$$k_1/k_2 = (m_2{*}/m_1{*})^{1/2} \, (1 + G(u)\Delta(u))$$

Harrison and Thode (62) report the value of $G(u)\Delta(u)$ for sulphite ion as 0.068 and 0.059 at 0°C and 25°C respectively. The quantity $(m_2^*/m_1^*)^{1/2}$ is evaluated by taking the ratio of the reduced masses of the two atoms being separated and is found to be 1.010. This gives the maximum value of

k_1/k_2 as 1.079 and 1.070 at 0°C and 25°C respectively.

In any case, it is clear that a better understanding of the physiological processes is required to account for these large isotope effects.

Reduction of Sulphate Study

Attempts to reduce sulphate ion by *Salmonella* were not successful. In only one case after a very long time sufficient H_2S was produced for analysis and the S^{34}/S^{32} ratio was nearly the same as the isotopic composition of the $SO_4^{=}$. The product was so small that one can not rule out the possibility of it arising from an impurity in the medium.

BIBLIOGRAPHY

(1) Boltwood, B. B., Amer. J. Sci., 22, 537 (1906).

(2) Boltwood, B. B., Amer. J. Sci., 24, 370 (1907).

(3) Soddy, F., Chem. Soc. Ann. Rep., 7, 285 (1910).

(4) Thomson, J. J., Proc. Roy. Soc. (London), A89, 1 (1914).

(5) Aston, F. W., Phil. Mag., 38, 709 (1919).

(6) Aston, F. W., Phil. Mag., 39, 449 (1920).

(7) Aston, F. W., Phil. Mag., 42, 436 (1921).

(8) Dempster, A. J., Phys. Rev., 11, 316 (1918).

(9) Dempster, A. J., Phys. Rev., 18, 415 (1921).

(10) Dempster, A. J., Phys. Rev., 20, 631 (1922).

(11) King, A. S. and Birge, R. T., Nature, 124, 127 (1929).

(12) Giaque, W. F. and Johnston, H. L., J. Am. Chem. Soc., 51, 1436 (1929).

(13) Naude, S. M., Phys. Rev., 36, 333 (1930).

(14) Urey, H. C., Brickwedde, F. G., and Murphy, G. M., Phys. Rev., 39, 164, 864 (1931).

(15) Rittenberg, D. and Urey, H. C., J. Chem. Phys., 2, 106 (1934).

(16) Farkas, L. and Farkas, A., Trans. Farad. Soc., 30, 1076 (1934).

(17) Urey, H. C., and Grieff, L. J., Am. Chem. Soc., 57, 32 (1935).

(18) Urey, H. C., J. Chem. Soc., 562 (1947).

(19) Washburn, E. W. and Urey, H. C., Proc. Natl. Acad. Sci. U. S., 18, 496 (1932).

(20) Eyring, H., Proc. Natl. Acad. Sci. U. S., 19, 78 (1933).

(21) Bach, F., Bonhoeffer, K. F., and Fajans, E., Zeit für Physik. Chimie., A168, 313 (1934).

(22) Farkas, L. and Farkas, A., Naturwiss, 22, 218 (1934).

(23) Urey, H. C. and Teal, G. K., Rev. Mod. Phys., 7, 34 (1935).

(24) Eidinoff, M. L., Trans. N. Y. Acad. Sci., 16, 76 (1953).

(25) Lindsay, J. G., McElcheran, D. E. and Thode, H. G., J. Chem. Phys. 17, 589 (1949).

(26) McMullen, C. C. and Thode, H. G., Mass Spectrometry edited by McDowell C.A., (1963) McGraw Hill.

(27) Krouse, H. R. and Thode H. G., Can. Jour. Chem. 40, 367 (1962).

(28) Brown, H. M. and Krouse H. R., Can. J. Chem., 42, 1971 (1964).

(29) Emeleus, H. J., James, F. W., King, A., Pearson, T. G., Purcell, R. H., and Brisco, H. V. A., J. Chem. Soc., 1209 (1934).

(30) Dole, M., J. Am. Chem. Soc., 57, 2731 (1935).

(31) Dole, M., Chem. Rev., 51, 263 (1952).

(32) Nier, A. O., and Gulbransen, E. A., J. Am. Chem. Soc. 61, 697 (1939).

(33) Murphy, B. F. and Nier, A. O., Phys. Rev., 59, 771 (1941)

(34) Thode, H. G., MacNamara, J., and Collins, C. B., Can. J. Res., B 27, 361 (1949).

(35) MacNamara, J., Fleming, W. H., Szabo, A., and Thode, H. G., Can. J. Chem., 30, 73 (1952).

(36) Vinogradov, A. P., Chupakhin, M. S. and Grinenko, V. A. Geokhimiia., 4, 3-9 (1956).

(37) Kulp, J. L. Ault, W. V., and Feely, H. W., Econ. Geol., 51, 139 (1956).

(38) Sakai, H., Geochim. Cosmochim. Acta., 12, 150 (1957).

(39) Tudge, A. P. and Thode H. G., Can. J. Res., B28, 567 (1950).

(40) Szabo, A., Tudge, A. P., MacNamara, J., and Thode, H. G., Science, 111, 464 (1950).

(41) MacNamara, J. and Thode, H. G., Research, 4, 582 (1951).

(42) Thode, H. G., Wanless, R. K. and Wallauch, R., Geochim. Cosmochim. Acta., 5, 286 (1954).

(43) Thode, H. G., Kleerekoper, H. and McElcheran, D. E., Research, 4, 581 (1951).

(44) Jones, G. E., Starkey, R. L., Feely, H. W., and Kulp, J. L., Science, 123, 1124 (1956).

(45) Kaplan, I. R., Rafter, T. A. and Hulston, J. R., N.Z.J. Sci., 3, 338 (1960).

(46) Harrison, A. G. and Thode, H. G., Trans. Farad. Soc., 54, 84 (1958).

(47) Harrison, A. G. and Thode, H. G., Trans. Farad. Soc., 53, 1648 (1957).

(48) Kaplan, I. R. and Rittenberg, S. C., Biochemistry of Sulphur Isotopes, edited by Jenson, M. L. (Proceedings of Natl. Sc. Found. Symp. - 1962).

(49) Kaplan, I. R. and Rittenberg, S. C., J. Gen. Microbiol., 34, 195-212 (1964).

(50) Nakai, N. and Jensen, M. L., Geochim. et. Cosmochim. Acta., 28, 1893 (1964).

(51) Bigeleisen, J., J. Chem. Phys. 17, 675 (1949).

(52) Eyring, H., J. Chem. Phys., 3, 107 (1935).

(53) Evans, M. G. and Polanyi, M., Trans. Farad. Soc., 31 875 (1935).

(54) Bigeleisen, J. and Mayer, M. G., J. Chem. Phys., 15, 261 (1947).

(55) Hirschfelder, J. O. and Wigner, J., J. Chem. Phys., 7, 616 (1939).

(56) Bigeleisen, J. and Wolfsberg, M., Advan. Chem. Phys., 1, 15 (1958).

(57) Wolfsberg, M., J. Chem. Phys. 33, 21 (1960).

(58) Brown, L. L. and Drury, J. S., Jour. Phys. Chem., 63, 1885 (1959).

(59) McKinney, C. R., McCrea, J. M. Epstein, S., Allen, H. A. and Urey, H. C., Rev. Sci. Inst., 21, 724 (1950).

(60) Wanless, R. K. and Thode, H. G., Rev. Sci. Inst., 30, 395 (1953).

(61) Hulston, J. R. and Shilton, B. W., New Zealand Jour. Sci., 1, 91 (1958).

(62) Harrison, A. G. and Thode, H. G., Trans. Farad. Soc., 53, No. 420, Part 12, (1957).

B29829